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## VARIATIONS IN GROWTH INDICES OF *VENUS MERCENARIA* L. FROM WIDELY SEPARATED ENVIRONMENTS OF THE ATLANTIC COAST<sup>1</sup>

BY CURTIS L. NEWCOMBE<sup>2</sup>, SARAH J. THOMPSON<sup>3</sup>, AND HERMAN KESSLER<sup>4</sup>

### Abstract

Linear and shell-weight indices of size in *Venus mercenaria* from three widely separated regions of the Atlantic Coast, namely, Gulf of St. Lawrence, Chesapeake Bay and North Carolina have been studied. The maximum variations revealed in the "b" values for the linear dimensions of *Venus* from the different regions are appreciable, whereas differences in the actual widths and thicknesses of corresponding lengths are not considered significant. The shell weights of specimens collected in the northern latitude of the Gulf of St. Lawrence are heavier than those from the warmer waters of the Chesapeake Bay and North Carolina. The length-shell-weight relations for the three regions are: Gulf of St. Lawrence,  $Wt. = 0.00000214 L^{3.003}$ ; Chesapeake Bay,  $Wt. = 0.00000171 L^{3.032}$ ; and North Carolina,  $Wt. = 0.00000108 L^{3.151}$ . No significant correspondence exists between linear growth dimensional ratios and known environmental influences, whereas shell weights seem to correspond with the temperature factor, an inverse relation being the result.

### Introduction

Comparatively few studies have been made on the variations in growth indices of pelecypod molluscs from widely separated regions. Work in this field has dealt chiefly with the growth of certain species collected from a single locality (2, 7). Several indices for expressing size and their relations have been definitely determined for several animals (Cf. 4, 6). Variations that occur in a single species throughout its geographical range have received very little attention.

In a comparative study of the linear and weight indices of *Mya arenaria* L., results have been obtained that indicate a close correspondence between temperature and shell weight, heavier shells being formed in the colder regions (5). With the idea of enlarging the available data dealing with this subject, upon which opinion is by no means unanimous, the present study was undertaken.

Variations in the linear indices of length, width, and thickness as well as differences in the shell weights of *Venus mercenaria* in the Gulf of St. Lawrence, Chesapeake Bay, and North Carolina regions have been investigated.

<sup>1</sup> Manuscript received October 6, 1937.

<sup>2</sup> A co-operative study from the Chesapeake Biological Laboratory and the Department of Zoology of the University of Maryland.

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Dry body weights of the soft parts were taken but are not included in this report owing to the uncertain nature of the effect of the preservative on the actual body weight. Brief mention is also made of the environmental factors that seem to distinguish the three regions.

The specimens were kindly provided by Dr. A. W. H. Needler, Assistant-in-Charge of the Prince Edward Island Biological Station; Dr. H. F. Prytherch, Director, U.S. Bureau of Fisheries Laboratory, Beaufort, N.C.; and Mr. G. T. Elliott, of Hampton, Va., for whose assistance we are very grateful. Acknowledgment is made to Dr. R. V. Truitt, Director of the Chesapeake Biological Laboratory, for his co-operation in all phases of the study.

### Methods

The linear dimensions, length, width, and thickness, were measured with a vernier caliper reading to 0.1 mm. The length measurement ( $L$ ) is the greatest anterior-posterior dimension; the width ( $W$ ) represents the greatest radius with the umbo as a centre; and the thickness measurement refers to the greatest distance between the two valves of the tightly closed animal placed in a lateral position.

All the collections were shipped to the laboratory in a living condition, dried to constant weight, and the individual shells weighed after the body parts had been removed. In all, 212 specimens were examined, the numbers from the individual regions being as follows: Gulf of St. Lawrence, 38; Chesapeake Bay, 38; and North Carolina, 136. Because of unfavorable field conditions at the time the collections were taken, it was not possible to obtain greater numbers from the Gulf of St. Lawrence and Chesapeake Bay regions.

Constants of the equations\*

$$(1) W = a + bL$$

$$(2) T = d + eL$$

expressing the relations of both width ( $W$ ) and thickness ( $T$ ) to length ( $L$ ) were obtained by the method of Lipka (3, p. 259). It was found that, within the length range studied, the relation between the logarithm of the length and that of shell weight is expressed by a straight line, hence the application of Huxley's power law, the form of the function being  $Wt = cL^k$ . The values of the constants  $c$  and  $k$  were calculated by Lipka's method.  $c$  is a constant denoting the value of  $Wt$  when  $L = 1$ , and has been referred to as the fractional coefficient. The constant  $k$  of the so-called simple heterogony formula represents the ratio of the relative growth rate of the shell weight to the relative growth rate of the length. The expression "relative growth rate" implies the rate of growth per unit dimension.

### Results

The growth dimensional ratios obtained for the North Carolina collection ( $N = 136$ ) are presented in Table I and may be readily compared with

\*  $a$  or  $d$  is a  $y$  intercept or a mathematical value of the width ( $W$ ) when the length ( $L$ ) is zero. This definition has no biological counterpart since it is necessary to have a length dimension before the width may be estimated.

$b$  or  $e$  is the slope of the line or the absolute increment of width corresponding to each unit increment of length.

corresponding ratios for the two remaining regions. It is seen that the "b" values (length-width) for the Chesapeake Bay ( $N = 38$ ) and Gulf of St. Lawrence ( $N = 38$ ) represent the maximum difference. A comparison of the length-thickness ratios of the same collections has shown that no significant difference in the "b" values obtains (Table I).

TABLE I  
CONSTANTS OF THE FUNCTIONS  $W = a + bL$ ,  $T = d + eL$  AND  $Wt = cL^k$  IN WHICH  
 $W$  = WIDTH,  $T$  = THICKNESS,  $Wt$  = WEIGHT, AND  $L$  = LENGTH

Dimensional ratios	Constants	Gulf of St. Lawrence	Chesapeake Bay	North Carolina
Length to width	$b$	0.931	0.772	0.852
	$a$	-2.876	2.274	1.395
Length to thickness	$e$	0.560	0.583	0.570
	$d$	0.378	-0.634	-0.631
Length to shell wt.	$k$	3.003	3.032	3.151
	$c$	0.00000214	0.00000171	0.00000108

Comparison of the actual widths and thicknesses corresponding to the same lengths for the three widely separated regions shows that the greatest difference exists between the Chesapeake Bay and the Gulf of St. Lawrence clams, namely 12% for specimens 80 mm. in length. Values for the North Carolina and Gulf of St. Lawrence specimens are very similar (Figs. 1, 2).

The  $k$  values, which represent the ratios of the relative growth rates of shell weights as compared with lengths for the different regions, show that a maximum variation (5%) exists between the Gulf of St. Lawrence and North Carolina regions (Table I). The ratio found for the former group is very close to that obtained for the Chesapeake specimens. A noticeable variation exists in the actual shell weight corresponding to a given length. The differences are more conspicuous in the smaller specimens. The maximum variation exists between the specimens from the Gulf of St. Lawrence and those from North Carolina, clams 20 mm. long from the former region being 32% heavier and those 50 mm. long being 12% heavier. The Chesapeake Bay specimens bear a close similarity to those of North Carolina, the smaller ones being relatively heavier and the larger ones somewhat lighter.

### Discussion

The data upon which this discussion is based were gathered from a study of specimens collected in the Gulf of St. Lawrence, Chesapeake Bay and North Carolina regions. The observations made are treated from the standpoint of regional variations in indices of size and in environmental conditions. There is little available information concerning the environmental factors operating in these regions. Temperature appears to be the factor that may

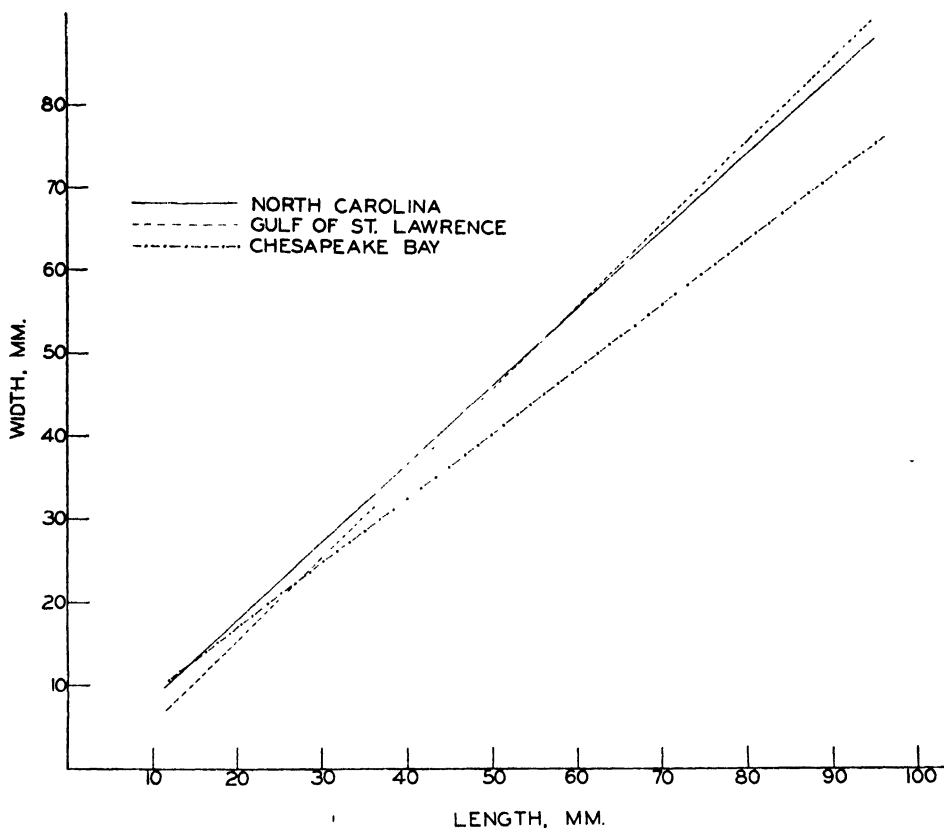


FIG. 1. Showing length-width relations of *Venus mercenaria* from three regions of the Atlantic Coast. Gulf of St. Lawrence,  $W = -2.876 + 0.931 L$ ; Chesapeake Bay,  $W = 2.274 + 0.772 L$ ; North Carolina,  $W = 1.395 + 0.852 L$ .

influence the growth indices of *Venus mercenaria* (Cf. 5). The variations in soil and salinity do not seem sufficiently great to warrant serious consideration. Mean daily water temperatures during July and August in the region of Malpeque Bay (Gulf of St. Lawrence) average about 20° C. in comparison with values of about 26° C. in the section of the Chesapeake where the collection was taken\* (1). Corresponding values for the North Carolina area are probably three degrees higher.

The results of this study seem to indicate a fairly close agreement with respect to the linear indices of the specimens studied. As is noted above, the "b" value for the length-width ratio of the Chesapeake collection is lower than that for the two remaining regions and seems to constitute one exception. Newcombe and Kessler (5) found that no significant variation exists in the linear growth dimensional ratios of *Mya arenaria* collected from different latitudes on the Atlantic Coast. This conclusion appears to lend support to the contention that a similar uniformity exists in *Venus mercenaria*.

\* Accurate records taken regularly are not available; hence values used are estimations based on irregular readings by different people during several seasons.

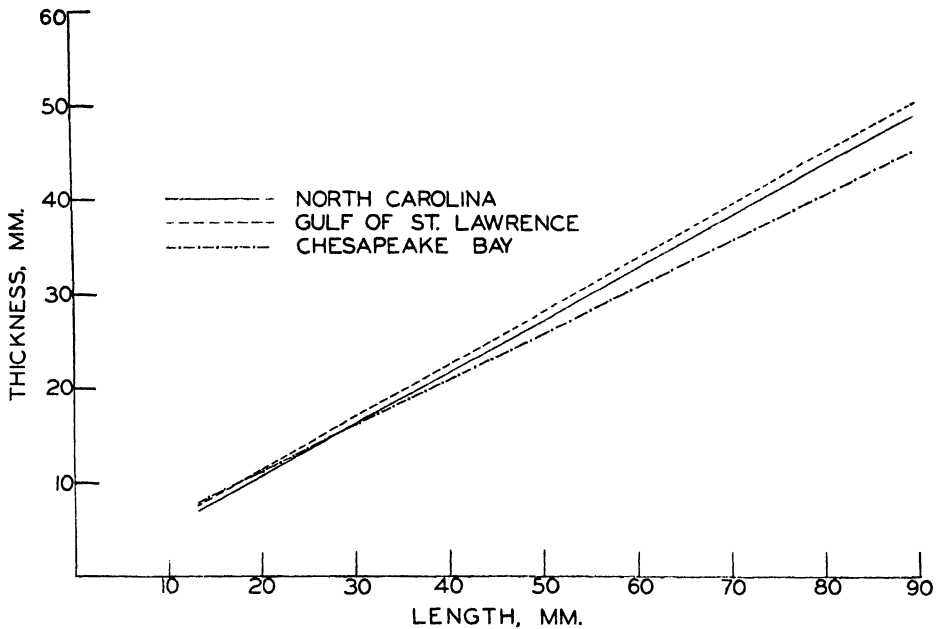


FIG. 2. Showing length-thickness relation of *Venus mercenaria* from three regions of the Atlantic Coast. Gulf of St. Lawrence,  $T = 0.378 + 0.560 L$ ; Chesapeake Bay,  $T = -0.634 + 0.583 L$ ; North Carolina,  $T = -0.631 + 0.570 L$ .

It seems significant that the shell weights of *Venus* collected in the northern latitude of the Gulf of St. Lawrence are heavier than those of the two southern regions. There is added interest in view of the fact that specimens of *Mya arenaria* grown in the cold waters of the Bay of Fundy possess heavier shells than those living in the warmer waters of the Chesapeake Bay. On the other hand, specimens of *Venus mercenaria mortensii*, inhabiting the warmer waters of the Florida coast and considered by some systematists to be the same as the Chesapeake Bay form, possess distinctly heavier shells. In view of this fact, the inverse relation between shell weight and temperature obtained in this study seems quite significant.

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## CONCERNING THE USE OF INDIRECT BIOCHEMICAL TESTS FOR THE DIAGNOSIS OF CHRONIC CONTAGIOUS MASTITIS<sup>1</sup>

BY C. K. JOHNS<sup>2</sup> AND E. G. HASTINGS<sup>3</sup>

### Abstract

Series of samples taken at consecutive milkings were analyzed to determine the reliability of indirect biochemical tests (chlorides, catalase and pH) for the detection of chronic contagious mastitis. It was found that infected quarters not infrequently yield normal milk while many non-infected quarters yield milk giving definitely abnormal reactions. Furthermore, the reactions to these tests frequently fluctuate widely from milking to milking for both infected and non-infected quarters.

These findings suggest the need of caution in the use of these tests as the basis for diagnosing mastitis infection, especially since the proportion of apparently normal animals showing abnormalities in the secretion is probably much larger in many herds than is generally appreciated.

These studies emphasize the value of examining a series of samples at consecutive milkings in order to obtain a true picture of the condition of a quarter. They also suggest that of the three tests studied, the catalase test appears to be the most sensitive indicator of infection.

The diagnosis of chronic contagious mastitis has received a great deal of attention during recent years, and numerous publications record the findings and opinions of workers in this field. Considerable emphasis has been placed by certain workers upon the value of various indirect biochemical tests (chlorides, pH, catalase, etc.) since these are much less laborious and time-consuming than the cultural demonstration of the presence of the streptococcus (*Str. agalactiae*) associated with this disease. Attempts have also been made to compare the accuracy of these indirect tests, although obviously the percentage of positive results from any one test will depend largely upon just where the line is drawn, for any constituent, between the normal and abnormal, as well as upon whether foremilk, middle milk or strippings are examined. The purpose of the present paper is to draw attention to certain objections to the use of these tests as diagnostic agents and particularly to warn against the inadvisability of relying upon such tests, alone or in combination, as the sole basis for diagnosis.

The data to be presented were obtained during studies of abnormal milk from young animals in the herds of the Central Experimental Farm, Ottawa, and the University of Wisconsin, Madison, which by repeated tests had been shown to be free from infection with *Str. agalactiae* or other known pathogens. A few cases of infection with *Str. agalactiae* or *Staph. aureus* were also included for comparative purposes.

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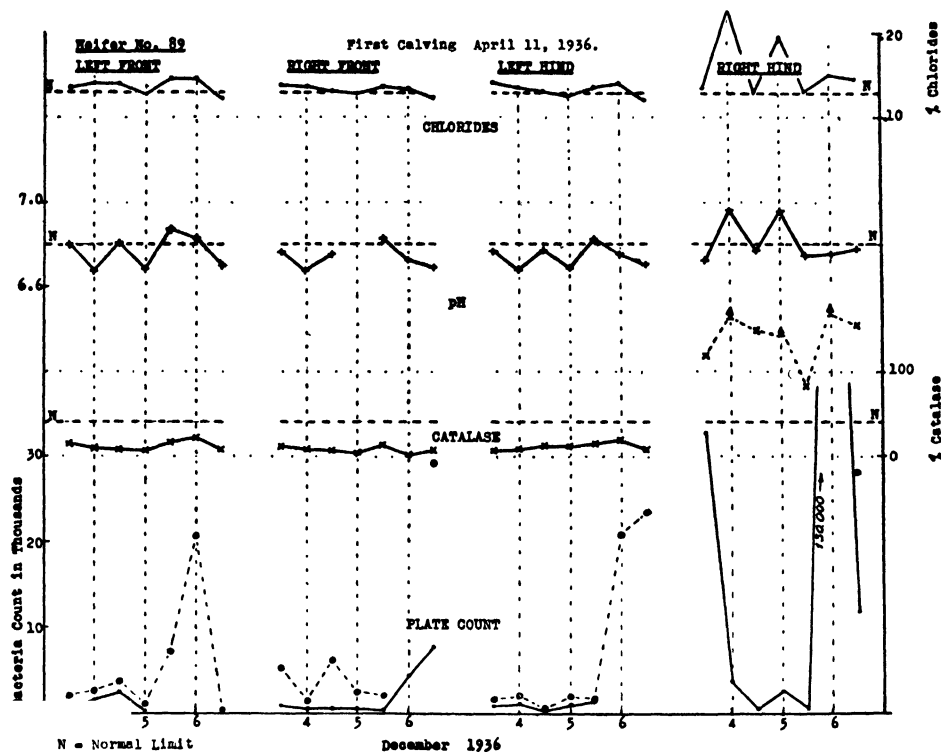


FIG. 1. Data on foremilk samples from seven consecutive milkings. Heifer No. 89. (Values for morning milkings on vertical dotted lines. Total counts indicated by broken line, counts excluding corynebacteria by solid line.)

## Methods

A number of methods were employed to detect the presence of streptococci, including the use of selective media, overnight incubation of milk with and without brilliant green, etc. Plate counts were originally made upon veal infusion agar but nutrient agar containing 0.5% tryptone (Difco) was mainly used as it yielded better growth.

The pH values were obtained electrometrically using a quinhydrone electrode. Chlorides were determined by titrating 5 cc. of undiluted milk with silver nitrate solution, using dichlorofluorescein as indicator (2). Catalase was determined by the method devised by one of us (E.G.H.); an ordinary glass tube of approximately 8 mm. internal diameter and 300 to 350 mm. length is corked at one end, and melted 2% plain agar introduced to a depth of 1 to 1½ inches. After the agar has solidified, the cork is removed and the agar plug blown one-third to one-half way down the tube. The mixture of milk (2 parts) and one per cent hydrogen peroxide (1 part) is now poured into the tube, the finger being placed at the lower end of the tube to prevent the agar plug from descending further. When the tube is filled, the cork is replaced, the tube inverted and a line marked at the upper level of the liquid. After

incubation for 24 hours at room temperature, the lengths of the columns of gas and liquid are measured and the amount of gas liberated calculated as follows:

$$\frac{\text{column of gas}}{\frac{2}{3} \text{ column of liquid}} \times 100 = \% \text{ oxygen liberated.}$$

Since none of the oxygen liberated is able to escape, as it does in the usual Smith fermentation tube, readings by the latter method are much lower and cannot be satisfactorily compared with those obtained by the method described above. Cheapness, ease of cleaning and a saving of space are other decided advantages of the Hastings tube method.

### Normal Values from Infected Quarters

While it is true that a large proportion of animals infected with *Str. agalactiae* yield milk which is high in pH, chlorides and catalase (or cell count, of which catalase is an indirect measure), especially in the first portion drawn, it is likewise true that normal values are not infrequently encountered even when appreciable numbers of streptococci are present. The data recorded for Heifer No. 89 are of interest in this connection. *Str. agalactiae* infection was detected in the right hind quarter during the fifth month of the first lactation period.

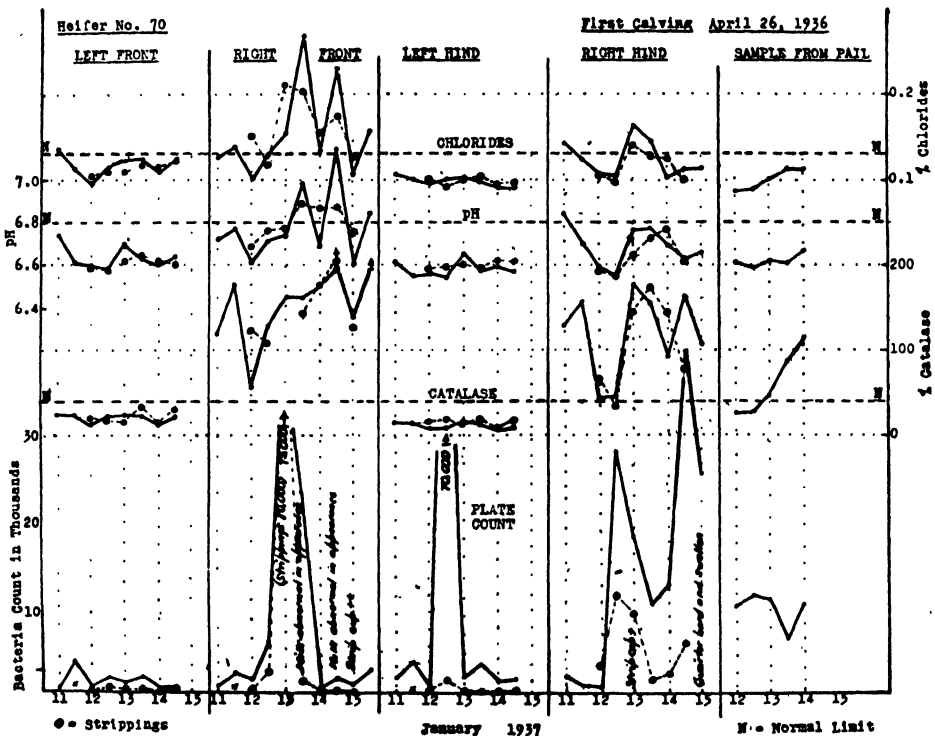


FIG. 2. Data on foremilk, strippings and pail samples, Heifer No. 70. (Values for afternoon milkings on vertical dotted lines.)

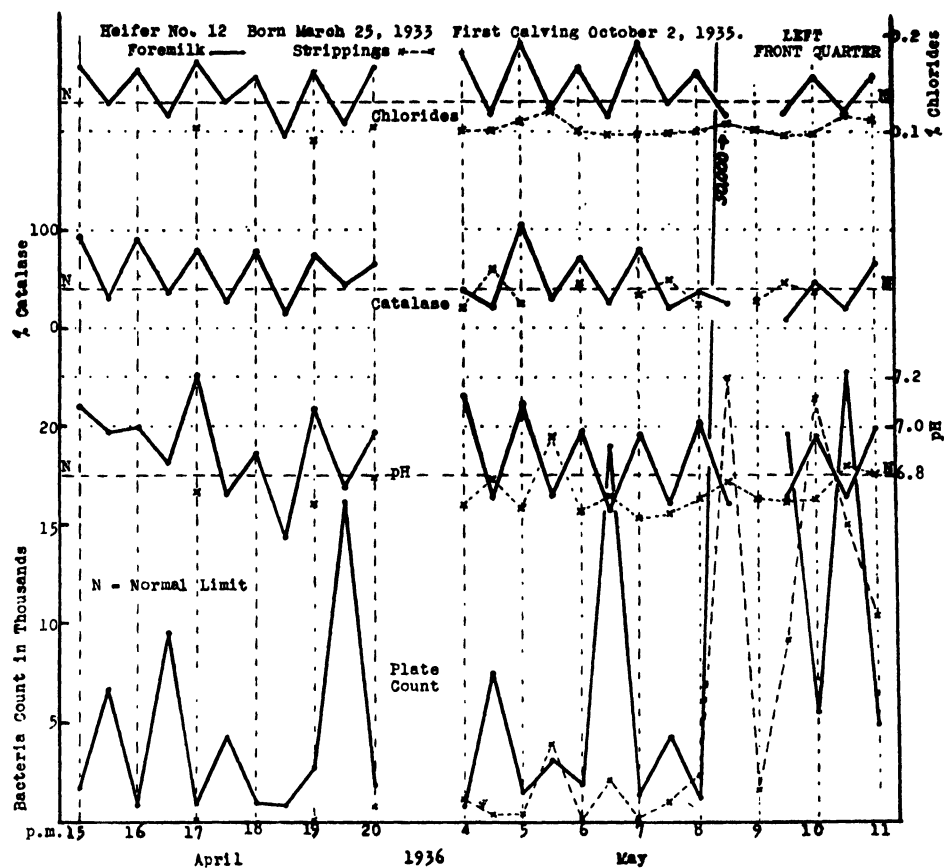


FIG. 3. Data on foremilk and strippings samples, left front quarter, Heifer No. 12. (Values for afternoon milkings on vertical dotted lines.)

Analyses of a series of foremilk samples from ten consecutive milkings disclosed little difference between this quarter and the three normal quarters, except for a higher level of values for catalase. A further series of samples from seven consecutive milkings was examined in the eighth month, the data from which appear in Fig. 1. At this time the general levels of values for chlorides and pH, as well as those for catalase, were definitely higher for the infected quarter. It will be observed, however, that there are marked fluctuations in the individual values for this quarter from milking to milking, samples from the afternoon milkings frequently being below the normal limit\* for pH. As in the former series, the difference between the infected and normal quarters is most marked with the catalase test, suggesting that this test possesses certain advantages over the chloride and pH tests in the early detection of this type of infection.

\* The limits tentatively established in these studies were: pH (quinhydrone) 6.8; chlorides 0.13%; catalase 40%. These values were found to be substantially equivalent for most of the animals studied.

Heifer No. 70 was carrying an infection with *Staph. aureus* in both right front and right hind quarters when she was studied during the ninth month of her first lactation period. She suffered an acute attack of mastitis in the right hind quarter on January 15. In Fig. 2 appear data for both foremilk and strippings samples. The contrast between the values for the normal and abnormal quarters is quite striking when a series of samples is examined, but it will be observed that a considerable number of normal values for pH and chlorides were obtained from the two infected quarters, and from the right hind quarter, even at a time when it was obviously hard and swollen. It will be observed that samples from the pail, representing the whole of the milk from the four quarters, were quite normal as judged by the chloride and pH values, while the catalase values increased markedly as the inflammatory process in the right hind quarter became more serious. Here again the catalase reaction appears to be a more sensitive indicator of infection than either the chloride or pH tests.

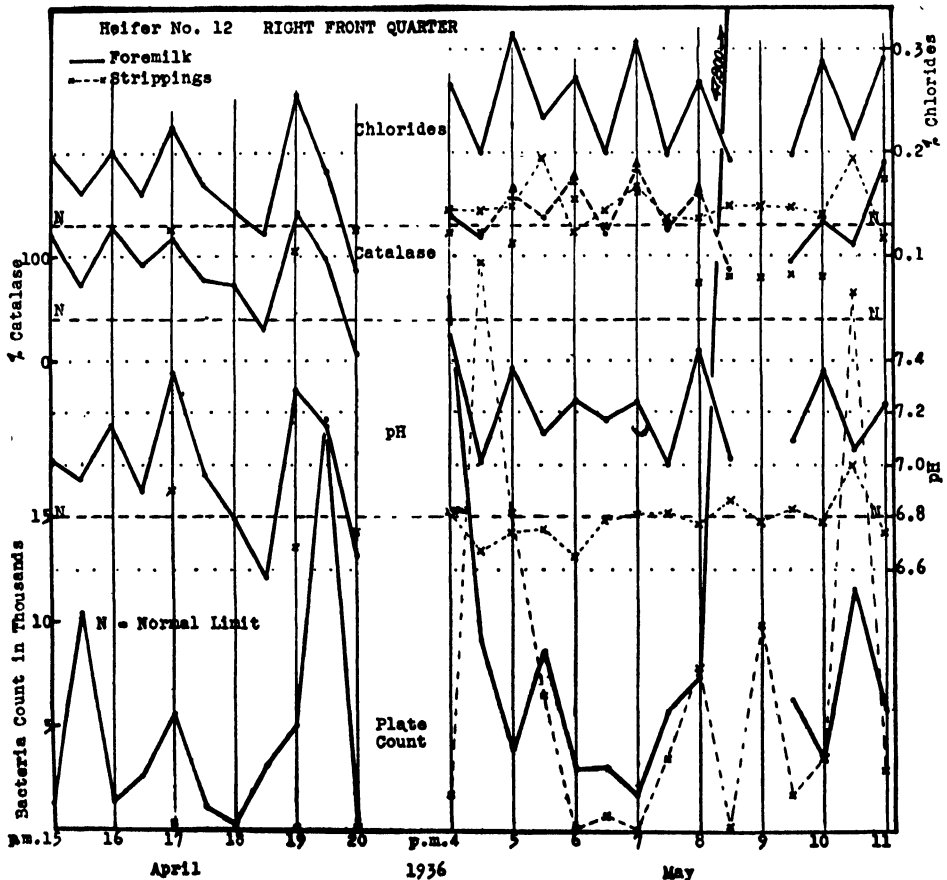


FIG. 4. Data on foremilk and strippings samples, right front quarter, Heifer No. 12. (Values for afternoon milkings on vertical dotted lines.)

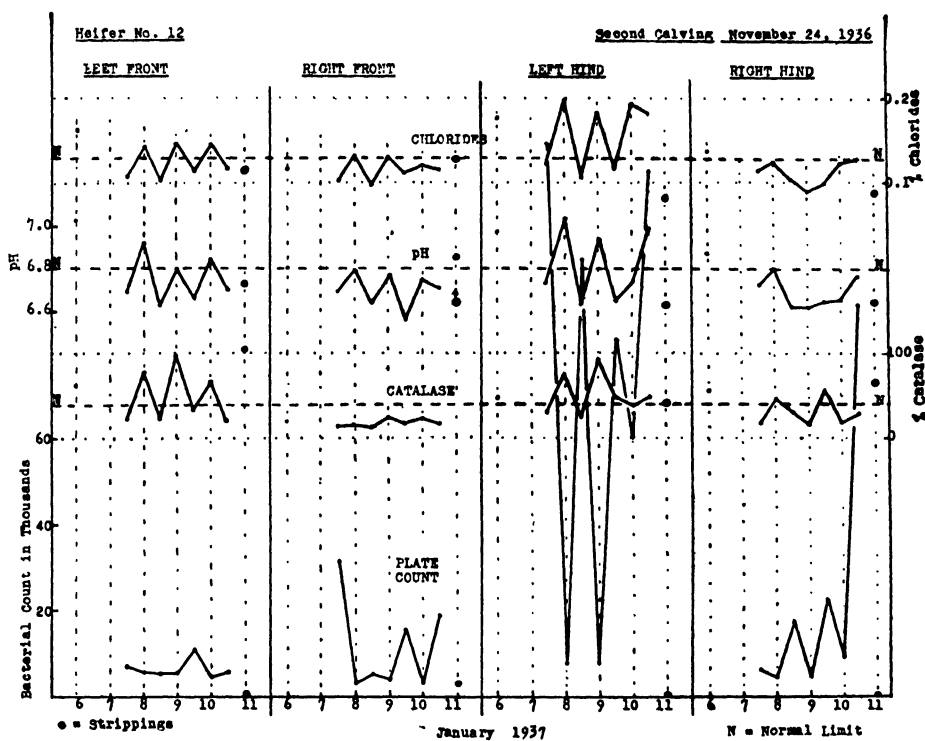


FIG. 5. Data on foremilk samples, Heifer No. 12. (Values for afternoon milkings on vertical dotted lines.)

### Abnormal Values from Non-infected Quarters

It will be noted that values for chlorides and pH slightly in excess of the normal limits were not infrequently encountered in the samples from the three normal quarters of Heifer No. 89 (Fig. 1). More striking illustrations of the occurrence of abnormal values in milk from quarters free from infection with any recognized pathogen are presented in Figs. 3 to 8. Fig. 3 records data from the left front quarter of Heifer No. 12 during the sixth and seventh months of the first lactation period. Similar abnormal values were encountered for three of the four quarters at this time. Of particular interest are the marked rhythmic fluctuations in biochemical values. Almost without exception the values are abnormal for the afternoon milkings and normal for the mornings, while an inverse relationship is displayed by the bacterial counts. Somewhat similar fluctuations were noted for the right front quarter (Fig. 4) although here the general level of values was definitely higher and few normal values were encountered. Similar pictures were obtained on several other occasions during the first and second lactation periods, of which Fig. 5 is fairly representative.

Another striking example of rhythmic fluctuation in biochemical values is afforded by Heifer No. 94. Samplings at ten consecutive milkings during

the ninth month of the first lactation period yielded the data presented in Fig. 6. Here again the values for the afternoon milkings were almost invariably much higher than those for the morning milkings.

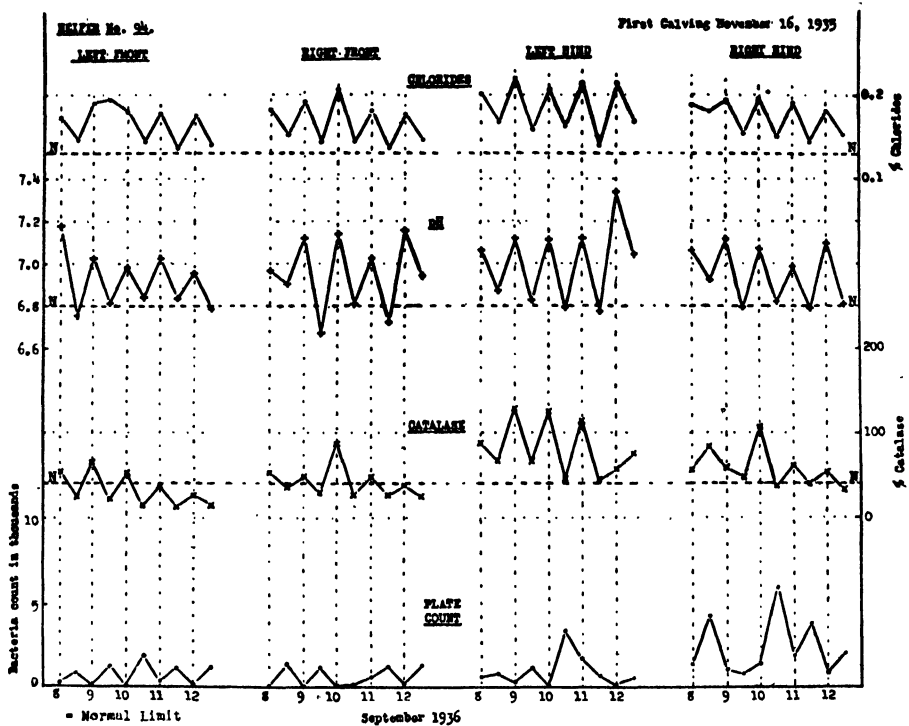


FIG. 6. Data on foremilk samples from 10 consecutive milkings, Heifer No. 94. (Values for afternoon milkings on vertical dotted lines.)

Even more marked fluctuations were encountered in the case of Heifer No. 18 (Hart Herd), data from which appear in Fig. 7. The contrast between the values for the normal (left front) and abnormal quarters is particularly striking. While few of the values for the abnormal quarters fall within the normal limit, it will be observed that there is a marked rhythmic fluctuation, values for the afternoon milkings being almost always higher than those for the morning milkings.

A somewhat different picture (Fig. 8) was obtained from Heifer No. 13 during the second month of her second lactation period. Here the regular rhythmic type of fluctuation is less evident; the general level of values is high, particularly for the front quarters, with tremendous variations from milking to milking. Here again normal values are not infrequently met with, especially on samples from the morning milkings. It will be noted that the values for all four quarters show a fair measure of agreement in their fluctuations.

### Discussion

The data presented above represent only a small portion of those obtained during the present studies. Just what proportion of apparently normal

animals in the average herd show abnormalities in the secretion cannot be estimated, but the findings in the Wisconsin Pasture Project herd reported by Hastings and Beach (1), together with those from further studies with other herds there, indicate that the proportion is not inconsiderable. That such abnormalities are unlikely to be due to *latent* infection with *Str. agalactiae* is shown by the following facts. More than seven hundred samples of foremilk, middlemilk and strippings from Heifer No. 12 were examined by a number of different procedures without once demonstrating the presence of the organism, while it has been repeatedly demonstrated without difficulty in samples taken concurrently from definitely infected animals. More than three thousand samples were similarly examined by Hastings and Beach without finding the streptococcus. The Main Herd at the University of Wisconsin has been free from infection with *Str. agalactiae* for more than two years, yet a survey made by one of us (C.K.J.) in March and April, 1937, showed that 39 of the 175 quarters sampled (or 21 of the 44 animals) gave abnormal reactions in at least one out of three samples taken at consecutive milkings. Miller (3) has reported similar findings from first-calf heifers showing no evidence of streptococcus infection. It seems likely therefore that this type of secretion abnormality, in which no recognized pathogen appears to be concerned, may be much more widespread than is generally

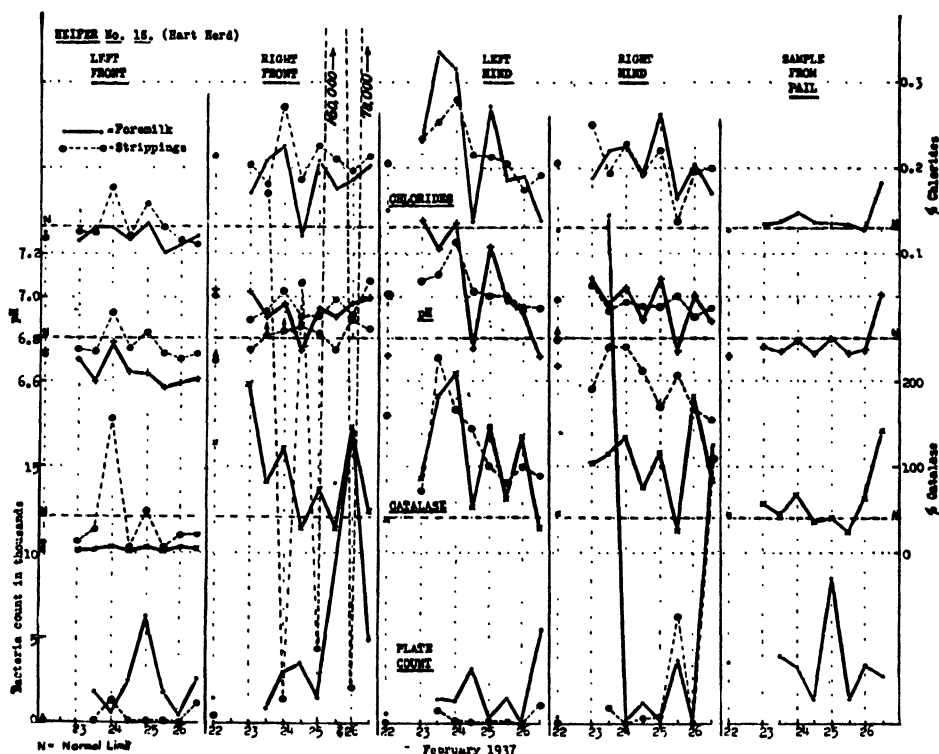


FIG. 7. Data on foremilk, strippings and pail samples, Heifer No. 18, (Hart Herd) (Values for afternoon milkings on vertical dotted lines.)

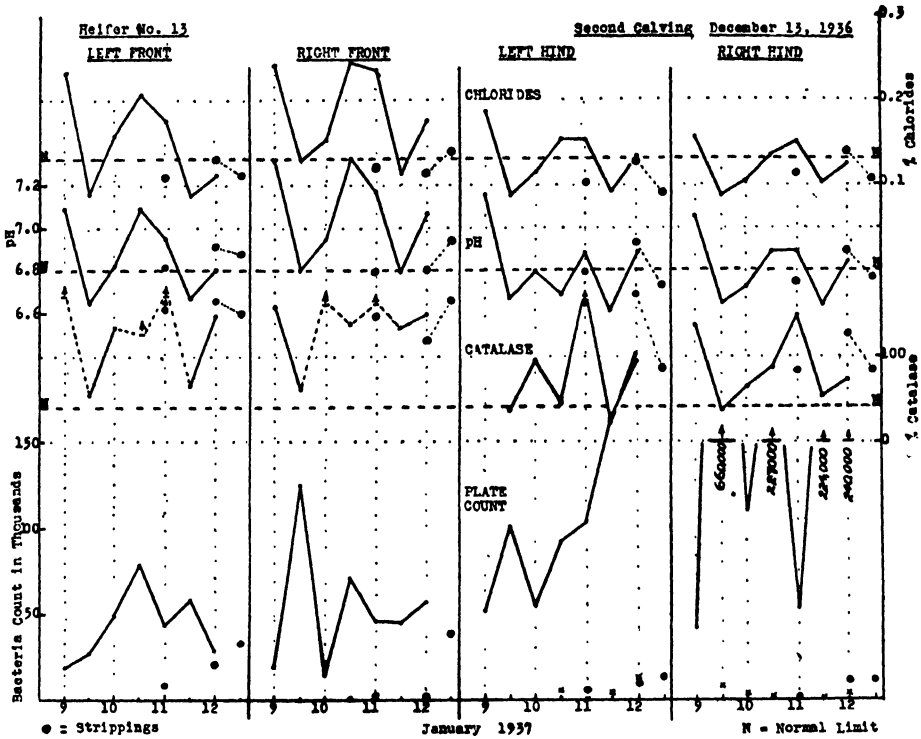


FIG. 8. Data on foremilk and strippings samples, Heifer No. 13. (Values for afternoon milkings on vertical dotted lines. Counts, excluding corynebacteria, indicated by crosses.)

realized, and suggests the need for greater caution in the use of indirect biochemical tests as the basis for diagnosing mastitis infection.

A striking feature of the present studies has been the demonstration of marked fluctuations, often of a regular rhythmic character, in the biochemical values for foremilk from milking to milking. Such fluctuations indicate the difficulty of attempting to determine the condition of a quarter by applying any type of test to a set of samples from a single milking. Obviously the verdict in many cases will depend entirely upon whether the samples were taken from the morning or afternoon milking. Similar fluctuations are frequently observed with the bacterial counts. Often low counts occur in samples with high biochemical values and high counts in samples with low values. It is therefore evident that a satisfactory picture of the condition of a quarter can best be obtained by the study of a series of samples taken at consecutive milkings, particularly where an attempt is being made to determine the relation between bacterial numbers and biochemical values.

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## THE RELATION BETWEEN BACTERIAL NUMBERS AND BIOCHEMICAL VALUES IN MILK FROM STREPTOCOCCUS-FREE QUARTERS<sup>1</sup>

BY C. K. JOHNS<sup>2</sup> AND E. G. HASTINGS<sup>3</sup>

### Abstract

An attempt has been made to determine whether abnormalities in the composition of the milk from streptococcus-free quarters are due to bacterial infection.

Four normal young animals whose previous history was known in considerable detail were selected for study. Series of samples from consecutive milkings were examined at intervals over a period of several months. Unfortunately none of the quarters studied showed more than a transitory rise in the level of catalase values during these detailed studies, so that the findings reported do not settle the question. Regular rhythmic fluctuations in counts from the foremilk were again noted, extremely high counts for the morning milkings alternating with low counts for the afternoon milkings.

The relation between bacterial numbers and abnormal composition of the foremilk is not a simple one. High biochemical values may accompany low count levels while extremely high counts may exert no apparent influence upon the composition of the milk at that time. Possibly a second agent in addition to bacteria is concerned.

More intensive studies of a single animal yielding milk with very high counts revealed higher biochemical values for strippings than for foremilk, those for catalase being surprisingly high. Despite the unusual nature of these findings, none of the four quarters became definitely abnormal during the fourteen weeks during which this animal was under observation, but subsequent routine samplings have shown that all four quarters must now be regarded as abnormal.

No one type of organism appears to be associated with changes in the composition of the secretion.

While the secretion yielded by cows infected with *Str. agalactiae* generally shows high values for chlorides, catalase and pH, similar abnormal secretions from significant numbers of streptococcus-free\* heifers have recently been reported by several workers (1, 3, 7). Since these "indirect" tests are widely used as diagnostic agents in attempts to control chronic mastitis, such findings suggest the need for extreme caution in their use.

Although the workers cited have all sought for the presence of some causative organism or organisms which might serve to explain the abnormal composition of the secretion, the search has so far been unsuccessful. Rarely

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\*In the light of the findings recently reported by Hucker (2) the term "streptococcus-free" indicates that mastitis streptococci were at no time demonstrated to be present in the milk.

does one encounter a quarter free from streptococci giving abnormal milk where a single bacterial species is constantly present in significant numbers. Too often the flora, both of foremilk and of strippings, is highly miscellaneous. The predominant types are various species of micrococci apparently identical with those occurring in milk consistently normal in composition.

It has previously been pointed out by one of us (3) that in at least one of the animals studied, there appeared to be some relation between total bacterial numbers and abnormal secretion. In the heifer in question the appearance of abnormal biochemical values (high chlorides, catalase and pH) in one quarter was preceded by a definite rise in the general level of bacterial counts. The studies to be described here deal with attempts to determine whether this always holds true or whether, as Wolf (9) maintains, the marked increase in bacterial numbers is due to the more favorable conditions furnished by the changed composition of the secretion.

It has been pointed out in a previous paper (3) that the most satisfactory picture of the condition of a quarter is obtained by the study of a series of samples taken at consecutive milkings. This method was followed in the present studies with animals carefully selected on the basis of their previous history.

## Part I

### PERIODICAL EXAMINATIONS OF MILK FROM FOUR NORMAL HEIFERS

Four animals, all in the first half of their second lactation period, were selected for study. Samples of foremilk from these animals had been examined\* at fortnightly intervals from their first calving up to the commencement of the present studies. In addition, at one milking each week, the milk from each individual quarter was collected separately by the use of a specially constructed milking machine. Details concerning the yield from individual quarters will be given as each animal is discussed.

#### *Sampling*

Commencing March 11, 1937, samples were obtained at five consecutive milkings. Three other similar series of samplings were conducted at short intervals, the last one commencing on April 11.

After discarding the first few streams, approximately 30 cc. of foremilk were drawn into a sterile container. No special precautions as to cleaning the udder and teats were employed. Samples were removed to the laboratory at once in the case of the afternoon milkings; approximately one hour elapsed at the morning milkings before the analysis commenced.

#### *Analysis*

Plate counts were made on a medium containing agar 2%, tryptone 0.5% and skim milk 1%, this medium having previously been found to be superior to veal infusion agar. Plates were counted after incubation for 48 hr. at 37° C.

\* These examinations were made by Dr. E. H. Peterson, to whom the authors wish to make acknowledgment for his courtesy in making his data available.

The catalase test was relied upon to indicate the relative normality of the milk from the quarters being studied, previous experience having shown it to be more satisfactory than the chloride or pH determinations (4). The modification devised by one of us (E.G.H.), in which the oxygen liberated is trapped beneath an agar seal in a long glass tube, was employed. Readings were made after 24 hours at room temperature (approximately 20-25° C.). On the basis of previous findings, a value in excess of 40% of gas expressed as a percentage of the volume of milk used in the test was considered to be definitely abnormal.

### *Findings*

*Heifer No. 3.* This animal began her second lactation period on October 31, 1936. No abnormalities were noted in the previous lactation period or up to the beginning of the present studies. During the first lactation period the left hind quarter yielded slightly less milk than the right hind, while the same has held true during the present lactation period. In addition, during the present period the left front quarter has yielded significantly less than the right front quarter.

Data obtained are presented graphically in Fig. 1\*. Normal values for catalase were encountered at each of the twenty milkings, samples from the right hind quarter excepted. The single high count on the left front quarter on March 12, a.m., appears to have been without influence upon the catalase values.

The right hind quarter, on the other hand, furnished samples with rather high catalase values during the second period of sampling (March 22 to 24). As the values had returned to normal by the following week it seems probable that the temporary rise in catalase values was associated with some physical injury. In view of the contention of Wolf (9) that abnormally high bacterial numbers are the result, rather than the cause, of abnormal secretion, it is of interest to note that in this case the counts remained low during and subsequent to the period when the catalase values were high.

There is nothing in either catalase values or counts to indicate any relationship between them and the relative milk production per quarter.

*Heifer No. 7.* During the first lactation period of this heifer, no consistently high values were noted, although the left front quarter occasionally yielded a sample with a high catalase value. Values for the left hind quarter were generally slightly higher than those for the other three quarters. No marked differences in production were noted for the four quarters. Nine days after the birth of the second calf on October 20, 1936, the milk was normal. Early in January 1937 the right front quarter suffered an acute attack of mastitis due to an infection with a coliform organism; at each subsequent sampling high values for catalase and chlorides were noted. The yield of milk from this quarter showed a sharp decline as a result of [this attack. The right hind quarter yielded about 25% more milk than the left hind during the present lactation period.

\*Values for the afternoon milkings appear on the dotted lines on all figures.

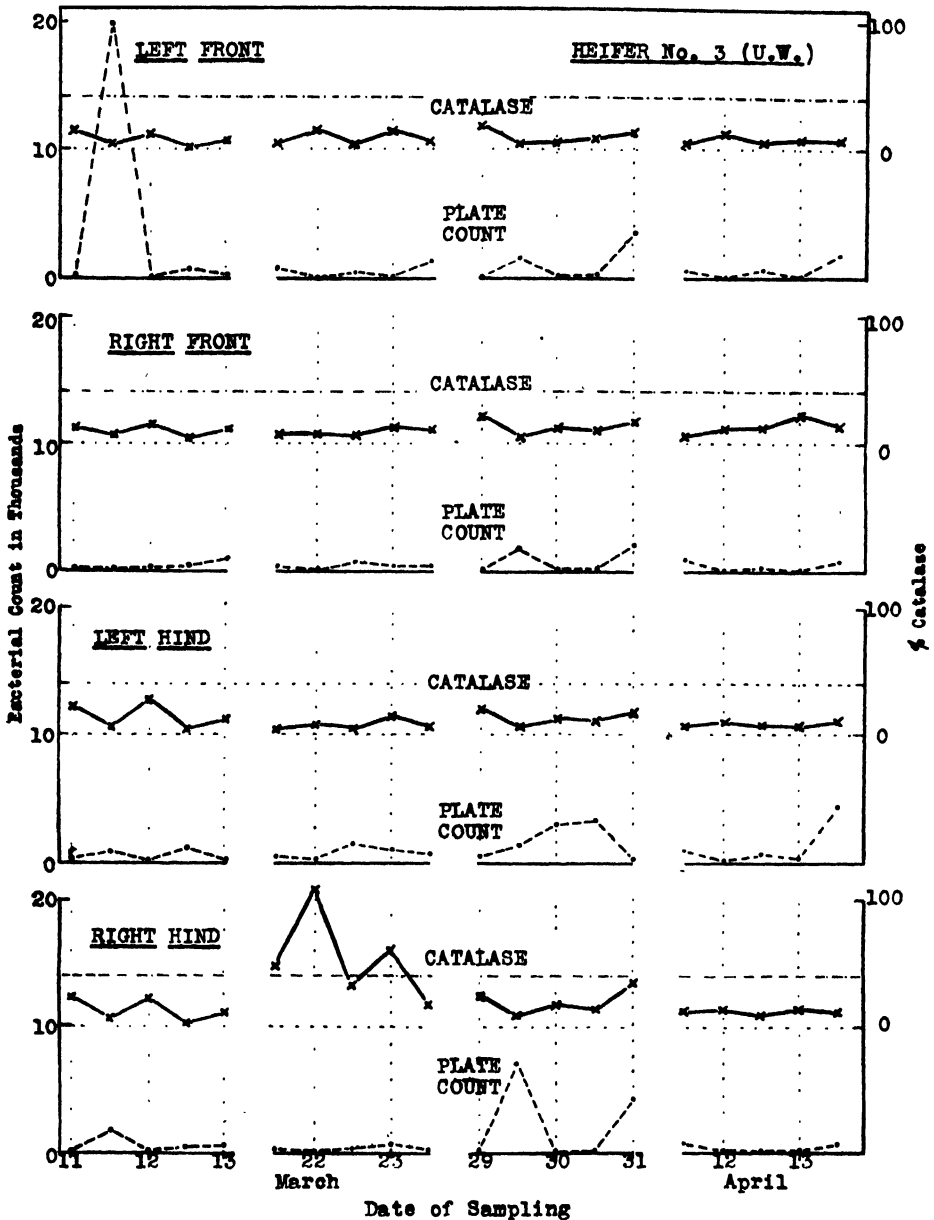


FIG. 1. Data on foremilk samples, Heifer No. 3, (U.W.)

From the data presented in Fig. 2 it will be observed that the influence of the acute attack of mastitis in the right front quarter was still being felt, although the general level of counts was comparable with those for the remaining quarters. The coliform organism was still present, the numbers fluctuating markedly from milking to milking. During the second period of sampling (March 22 to 24) there was a "flare-up" of the previous infection, large

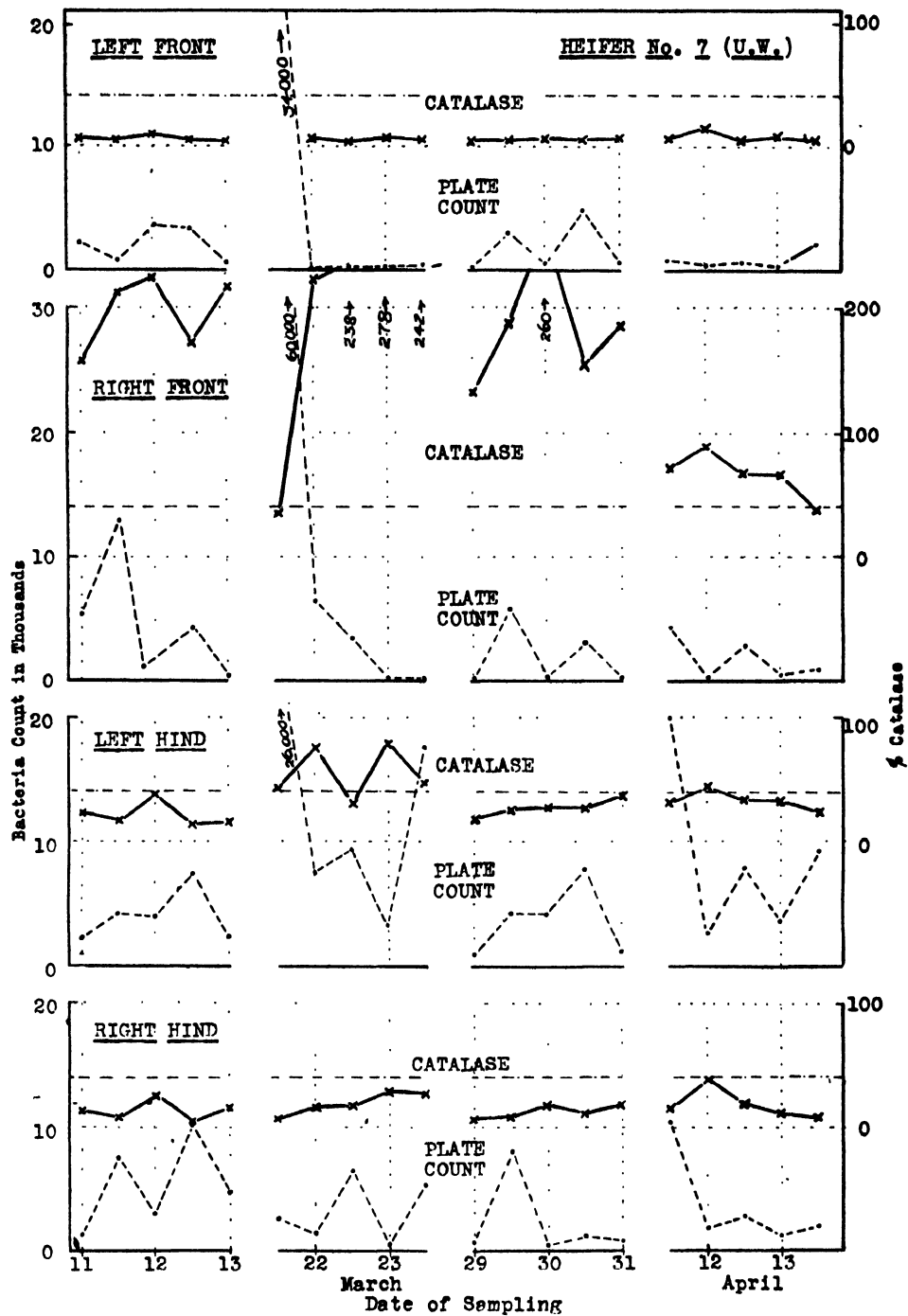


FIG. 2. Data on foremilk samples, Heifer No. 7, (U.W.)

numbers of the organism concerned being present in the sample drawn on the morning of March 22. The quarter was hard and the milk obviously abnormal. The catalase values rose sharply at this time, remaining at a very high level for several milkings after the count had declined. High catalase values accompanied by reasonably low counts appeared during the following week, while in the final series (April 12 to 14) the values were significantly lower, although still above the normal limit.

No values in excess of 40% were obtained for samples from the left front and right hind quarters but the left hind quarter showed a somewhat higher level of values throughout. During the second period of sampling, four of the five samples from this quarter were above the normal limit, while the level of counts was also definitely higher. It will be recalled that this quarter showed somewhat higher values for catalase during the first lactation period. Whether these higher levels were "normal" for this quarter or whether there was some slight disturbance of function cannot be decided on the basis of the information at hand.

It will be observed that bacteria counts were almost always higher for the morning samples while catalase values were generally higher at the afternoon milkings.

Excluding the definitely infected right front quarter, it appears that among the other three quarters the level of catalase values is in fairly close agreement with the level of bacteria counts. The occurrence of reasonably low counts concurrently with extremely high catalase values in the case of the right front quarter may serve to explain the finding of abnormal secretion from apparently normal udders where the bacteria counts are lower than might be expected. Such cases may be due to the presence of large numbers of bacteria in the udder at some previous time.

*Heifer No. 10.* No consistent abnormalities were noted during the first lactation, although the right front quarter suffered from an acute attack of mastitis on August 20, 1936, at which time a Gram-positive streptobacillus was isolated. Five days later the organism was no longer present in the milk and the composition rapidly returned to normal. Milk yields per quarter were comparable throughout the lactation period.

This animal commenced her second lactation period on November 23, 1936. A single abnormal catalase value of 90% was noted for the right hind quarter on January 3, but this was followed by normal values.

From the data presented in Fig. 3, it will be noted that the general levels of catalase values for the right front and right hind quarters were somewhat higher than those for the remaining quarters, with an occasional value in excess of 40%. Since the production by the right front quarter was comparable to that by the left front, while the right hind quarter yielded about one-fifth less than the left hind, there seems to be no clear connection between the level of catalase values and yield of milk.

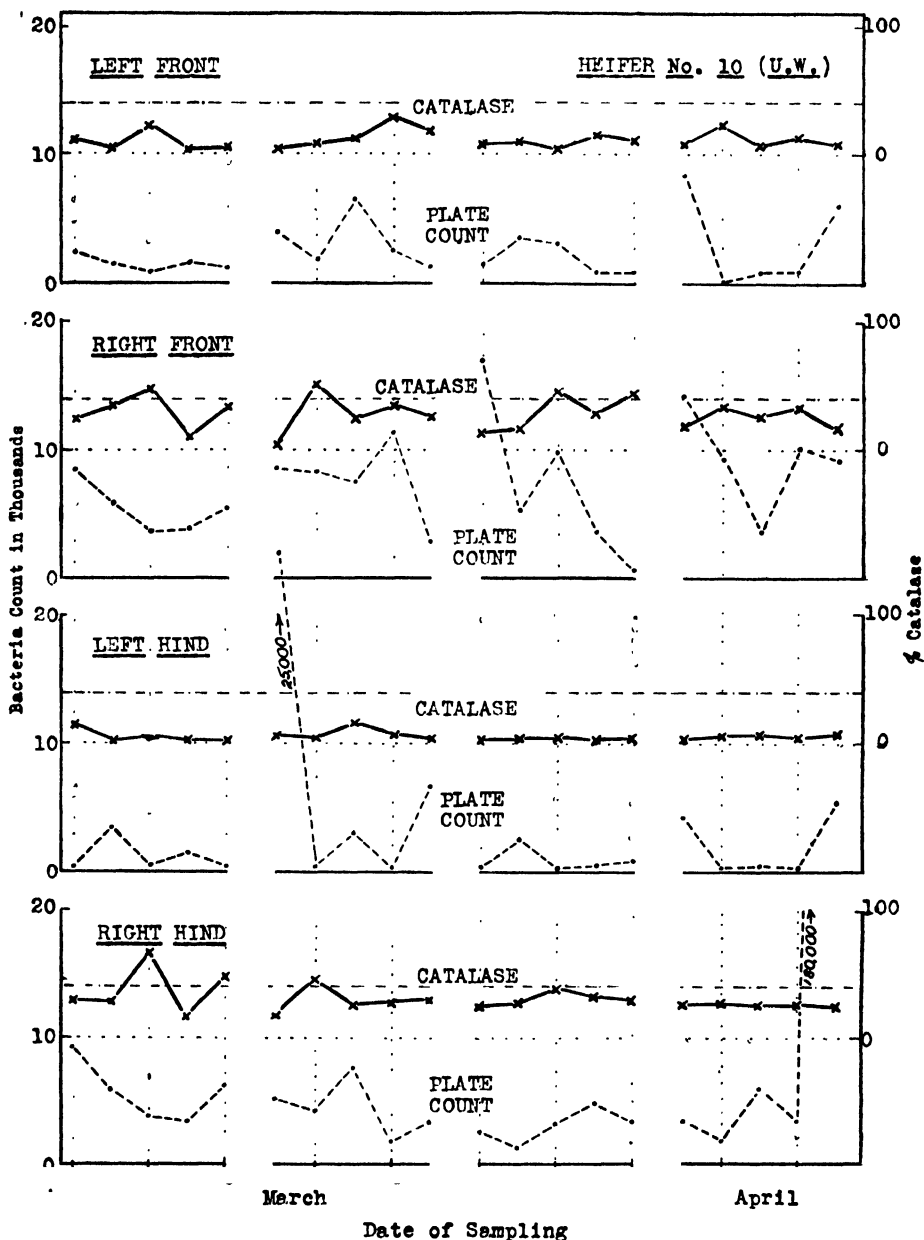


FIG. 3. Data on foremilk samples, Heifer No. 10, (U.W.)

As in the case of heifer No. 7, there is a fairly close agreement between the levels of catalase values and of bacteria counts for the various quarters.

*Heifer No. 12.* During the first lactation period of this heifer the two front quarters showed slightly higher values for chlorides and catalase than the

hind quarters, those for the right front quarter being the more abnormal. The right hind quarter produced over 20% more milk than the corresponding quarter during this period.

The second freshening occurred on February 8, 1937. Fairly normal values were encountered a week later, and these continued up to the time of the present studies. The right hind quarter is again producing more milk than the left hind quarter.

The unusually high counts obtained made it necessary in graphing the data to employ a scale in which each unit represents five times as many bacteria as those used for the data concerning the other three heifers. This should be borne in mind when comparing the data presented in Fig. 4 with those in the three preceding figures. In addition to the high counts, samples from all four quarters were characterized by a highly miscellaneous flora, no single type appearing in sufficient numbers to attract attention. Micrococci were most common, although tiny Gram-negative cocco-bacilli were occasionally isolated from all four quarters.

No abnormal catalase values were encountered, except from the left hind quarter upon two occasions. The general level of values for this quarter was definitely higher than those for the remaining three, while extremely high counts were obtained upon three occasions during the last two sampling periods. The enormous fluctuations in counts from milking to milking, with counts higher at the morning milkings, exhibit a regular rhythmic fluctuation similar to that previously noted in a number of other animals (4). Similar but less marked fluctuations were observed for the other three quarters.

The general high level of counts, with occasional extremely high counts, without any marked influence upon the catalase values, suggested the advisability of investigating the condition of the strippings. Accordingly, samples of both foremilk and strippings were obtained at the last three milkings (April 13 and 14) and analyzed for catalase, chlorides, pH and total count. Unfortunately, in five instances the sample of strippings was not large enough to enable all of these tests to be made. The data obtained appear in Table I. The contrast between the catalase values for the strippings and foremilk samples is most surprising. (In only one other animal whose foremilk and strippings have been studied has anything resembling this condition been encountered. In this case three of the four quarters were definitely abnormal; two of these and the remaining normal quarter all showed somewhat higher values for catalase on the strippings). While the pH values for strippings were all slightly higher than those for foremilk, the differences were of much smaller magnitude, with only two in excess of pH 6.8. Differences in chloride content were even smaller. These data, together with the occasional very high counts on strippings samples, indicated that the bacteria were not confined to the lower portions of the udder as in the cases reported by Little and Foley (6). It was believed that this animal was worthy of further study, conse-

quently further periodical samplings were conducted over the period May 3 to June 16. These are reported upon in the following section.

TABLE I  
DATA ON SAMPLES OF FOREMILK AND STRIPPINGS  
(HEIFER No. 12 (U.W.) APRIL, 1937)

—	Left front	Right front	Left hind	Right hind
<i>Counts</i>				
13th a.m. F	3,700	2,520	145,000	4,500
S	630	9,200	30	640
p.m. F	460	1,680	1,700	2,600
S	21,100	900,000	1,720	90
14th a.m. F	5,500	160,000	160,000	56,400
S	9,800	5,200	910	6,000
<i>Catalase</i>				
13th a.m. F	11	6	27	5
S	178	122	184	81
p.m. F	15	14	26	13
S	157	—	251	55
14th a.m. F	10	9	21	10
S	—	133	79	59
<i>pH</i>				
13th a.m. F	6.46	6.52	6.53	6.50
S	6.79	6.61	6.68	6.68
p.m. F	6.55	6.60	6.60	6.57
S	6.70	—	6.86	6.62
14th a.m. F	6.56	6.56	6.56	6.56
S	6.93	6.76	6.67	6.67
<i>Chlorides</i>				
13th a.m. F	0.101	—	0.100	—
S	0.120	—	0.095	0.112
p.m. F	0.100	0.105	0.105	0.097
S	0.119	—	—	0.100
14th a.m. F	—	0.125	0.115	0.105

F—Foremilk. S—Strippings.

## Part II

### FURTHER DETAILED STUDIES WITH HEIFER No. 12 (U.W.)

The unusual nature of the findings reported for this heifer suggested that further detailed studies of this animal might be more profitable than a continuation of those described in Part I. Arrangements were therefore made to analyze samples of both foremilk and strippings from five consecutive milkings at several periods during April, May and June, 1937. Determinations were made of chlorides and pH in addition to catalase and plate counts.

### Methods

The collection of samples, plating, and catalase determinations were conducted in the manner previously described. Chlorides were determined by titrating 5-cc. portions of the sample (diluted with 20 cc. of distilled water) with silver nitrate solution, dichlorofluorescein being used as indicator (5). Values in excess of 0.13% were regarded as abnormal. The pH values were determined electrometrically with the quinhydrone electrode, a value of 6.8 being taken as the upper limit for normal milk\*.

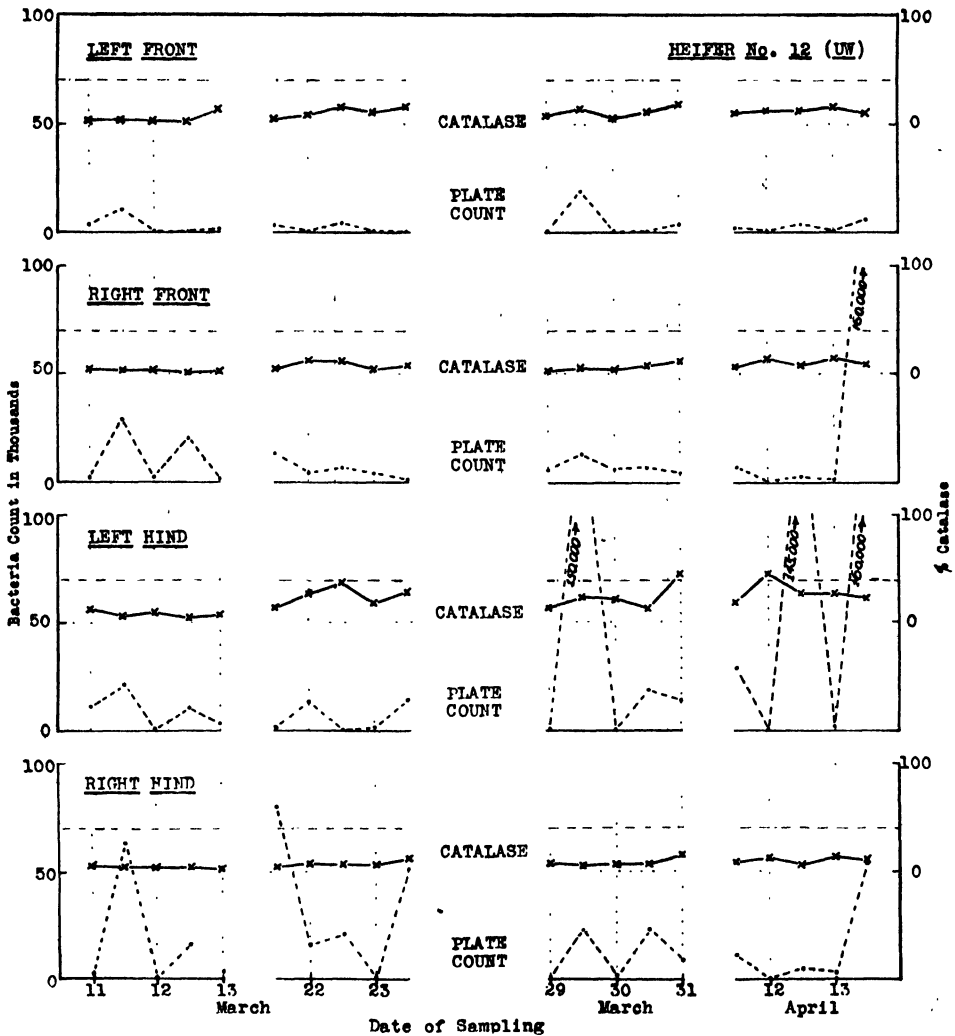


FIG. 4. Data on foremilk samples, Heifer No. 12, (U.W.)

\* These limiting chloride, catalase, and pH values for normal milk were based upon previous studies (4) where in the majority of cases these values were found to be substantially equivalent.

### Findings

The data for each quarter are presented in Figs. 5 to 8. It will be observed that the differences in values between foremilk and strippings were most pronounced in the case of catalase; chloride values showed the least differences, while pH values showed slightly greater differences than chlorides. Differences of similar magnitude have never been noted in the few other animals in which a study of both foremilk and strippings samples has been made (4); except for catalase values, which were sometimes slightly higher, both normal and abnormal quarters nearly always showed lower values for strippings.

While all four quarters showed this difference, it was less marked in the right hind quarter, which also showed a lower general level of values by all three tests. It will be recalled that this quarter was producing about 20% more milk than the left hind quarter.

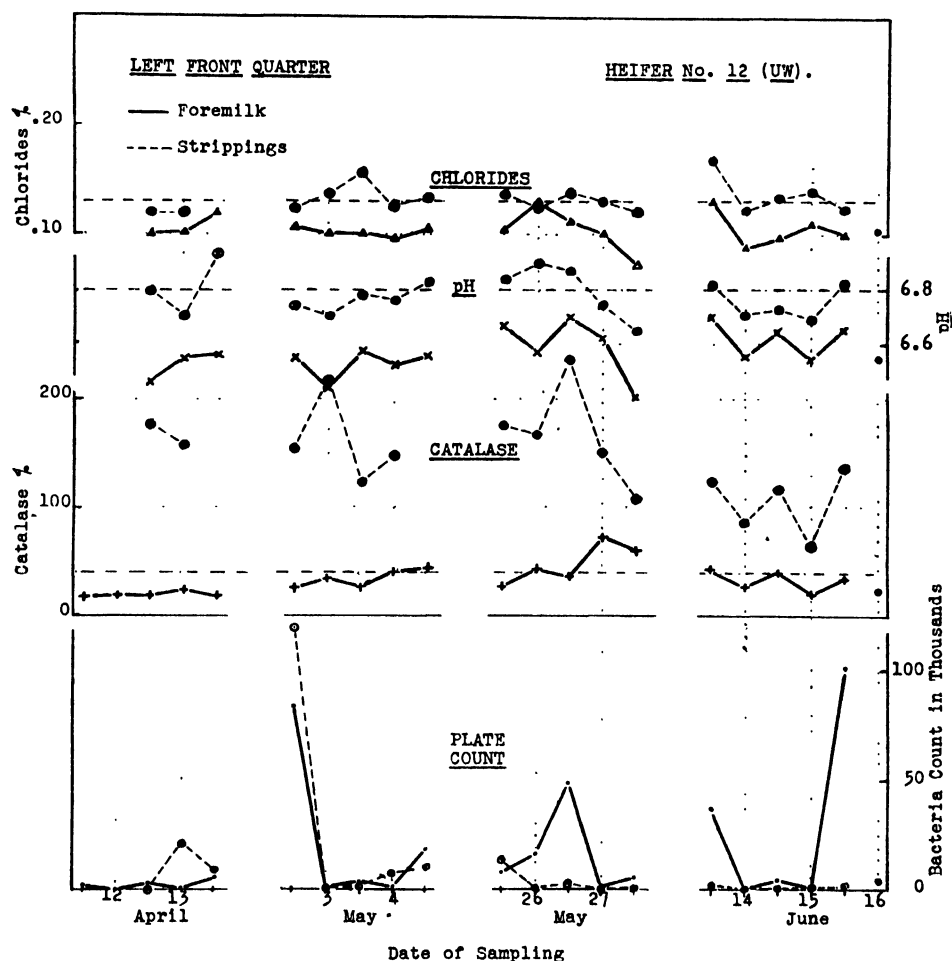


FIG. 5. Data on foremilk and strippings samples, Heifer No. 12 (U.W.)

In order to obtain some indication of whether the values for foremilk or strippings were more truly representative of the entire quantity of milk secreted by any one quarter at a given milking, samples of the latter were secured at the afternoon milking of June 16 when the production per quarter was being determined by the use of the special milking machine. The data obtained are indicated on the figures by solid dots. It will be observed that the values, especially those for catalase, were in good agreement with those for the foremilk, only one value (catalase for the right front quarter) being slightly in excess of the normal limits adopted in these studies.

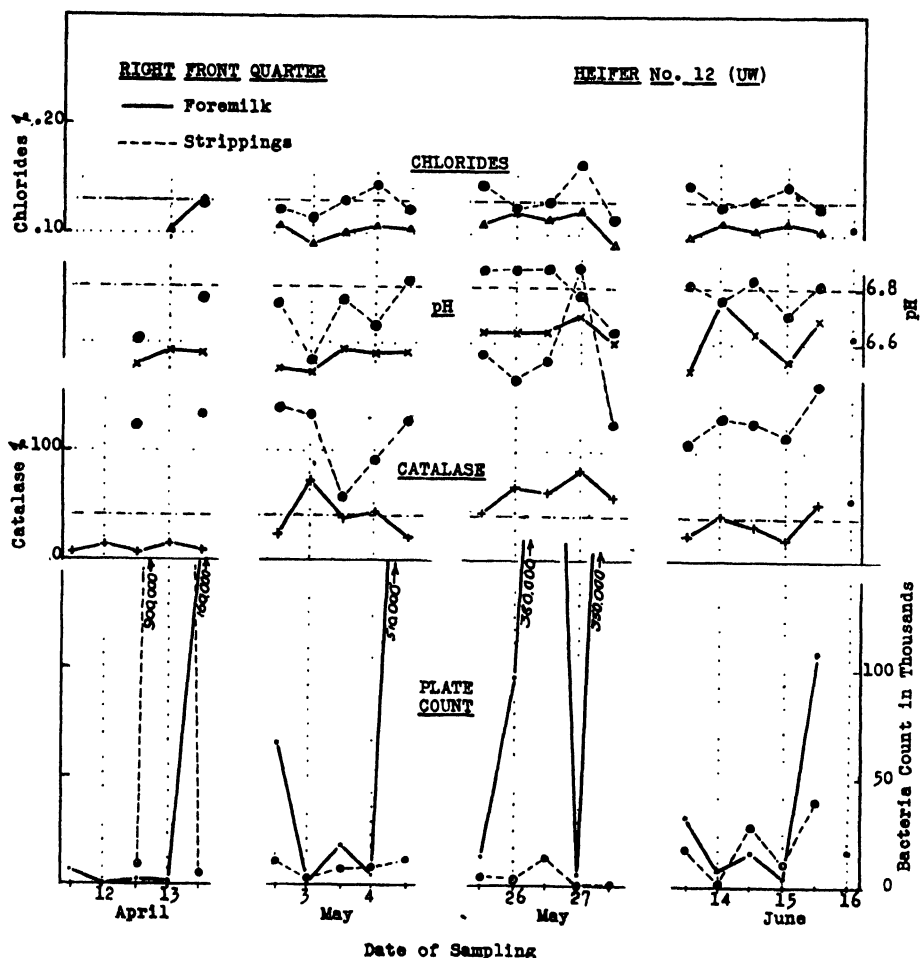


FIG. 6. Data on foremilk and strippings samples, Heifer No. 12 (U.W.)

The close agreement in direction and degree of fluctuations in values for chlorides, pH and catalase, which was so striking a feature in the majority of animals previously studied (4), was lacking here. Nor did the values for the various quarters fluctuate together as was often the case, while values for

foremilk and strippings appeared to fluctuate independently and showed little or no relation to fluctuations in bacteria counts. The gradual rise in the levels of values for foremilk up to the period of May 26 to 28 suggested that all four quarters would soon become definitely abnormal, but at the subsequent series of samplings on June 14 to 16 a decline was noted. The departure of the senior author a few days later unfortunately brought these observations to a close. However, routine sampling at the afternoon milking at fortnightly intervals since that time by Dr. Peterson has shown that the general levels of values for chlorides and catalase have increased fairly steadily for all four

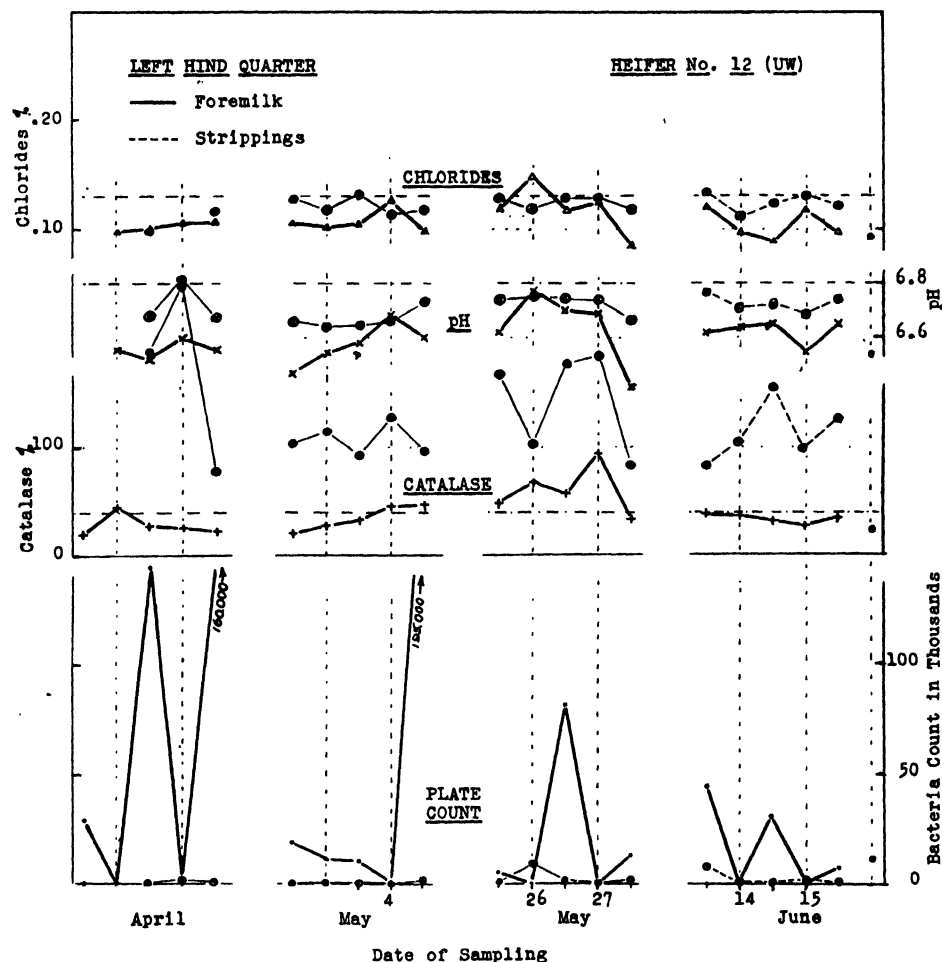


FIG. 7. Data on foremilk and strippings samples, Heifer No. 12 (U.W.)

quarters (Table II) so that none could now be regarded as normal. The right front quarter displays the greatest degree of abnormality and the right hind the least. Production for the right hind quarter is over 40% greater than for the left hind, while for the two front quarters production is fairly uniform.

This animal yielded 34.1 lb. of milk on September 6, 1937, seven months after freshening. Bacteria counts from samples taken at the *afternoon* milkings on August 16 and September 13 were fairly low, those for the right front quarter alone exceeding 225 per cc. (4,200 and 3,000 per cc.). This is in line with the findings reported above, high counts being rarely encountered except at the morning milkings.

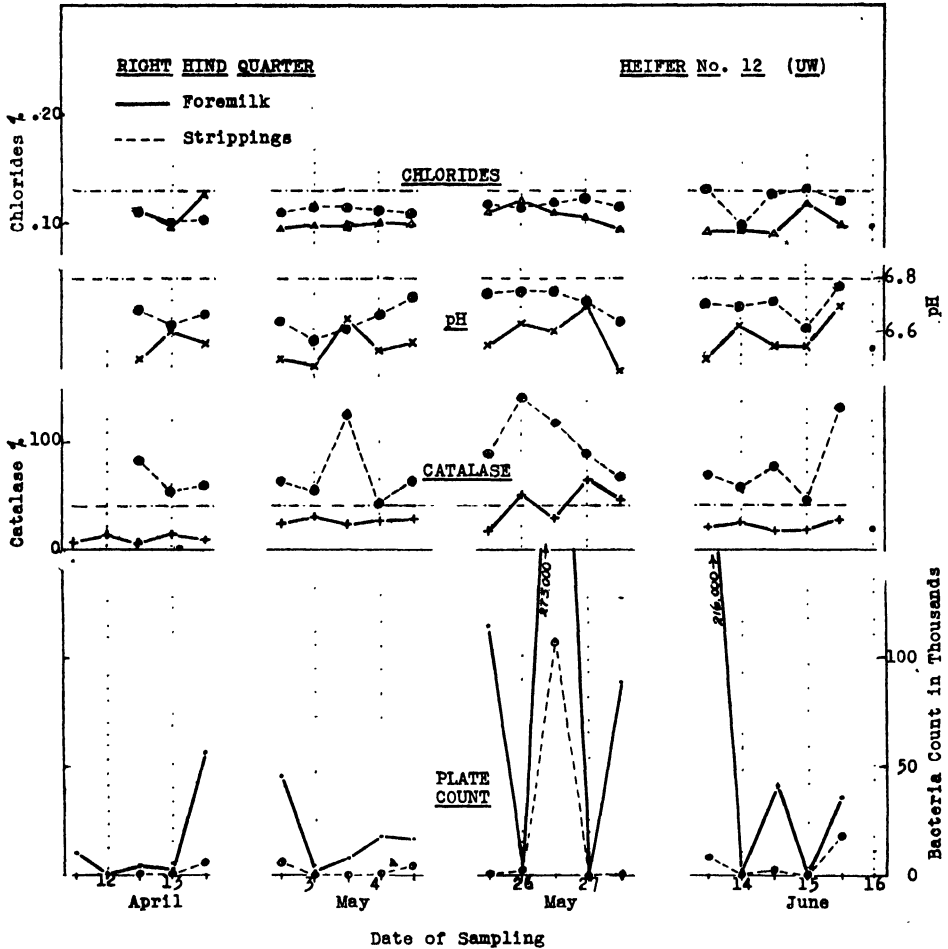


FIG. 8. Data on foremilk and strippings samples, Heifer No. 12 (U.W.)

While, in the studies reported in Part I, there was some indication of a correlation between the levels of bacteria counts and of catalase values for the left hind quarter, there was less evidence in this direction from these later studies. This quarter, with no higher general level of counts than the right hind, showed a greater degree of abnormality in secretion, especially in the strippings, while the right front quarter, in spite of a number of extremely high counts and the highest level of counts of the four quarters,

showed only a slightly greater degree of abnormality in the secretion than the left front quarter with a much lower count level. However, the right front quarter later developed a greater degree of abnormality in the secretion, as indicated in Table II.

TABLE II  
SUMMARY OF DATA FROM BI-WEEKLY ROUTINE SAMPLING OF FOREMILK OF HEIFER NO. 12 (U.W.)  
AT AFTERNOON MILKINGS, JUNE 22-SEPTEMBER 13, 1937

	Catalase, %			Chlorides, %			Milk production, average per milking
	Average	Maximum	Minimum	Average	Maximum	Minimum	
Left front	58.4	98	31	0.185	0.200	0.171	8.34
Right front	97.4	126	63	0.200	0.225	0.176	8.14
Left hind	54.8	83	36	0.176	0.187	0.167	8.09
Right hind	42.4	61	26	0.169	0.178	0.153	11.63

### General Discussion

With the exception of Heifer No. 12, the data acquired during these studies have not thrown much light upon the point at issue since the majority of the quarters previously regarded as normal have shown no indications of abnormality other than an occasional temporary rise in catalase values. However, Heifer No. 12, which at the start gave some extremely high counts, later became definitely abnormal in all four quarters, suggesting that large numbers of bacteria in the glands may have influenced the secreting tissue sufficiently to change the composition of the secretion.

In the case of the animals reported upon in this paper it is evident that the fairly definite relationship between fluctuations in bacterial numbers and in composition of the secretion which Steck (8) has described in his so-called "latent infection" does not hold. High values for catalase may be encountered along with low counts, as noted for the right hind quarter of Heifer No. 3. On the other hand, extremely high counts may occur with little or no concurrent evidence of abnormality as in the case of Heifer No. 12, although such high counts may be followed by abnormalities in the secretion at a later date. It would indeed be surprising if such enormous numbers of bacteria could be present in the gland for considerable periods without appreciably affecting the composition of the secretion. It must of course be recognized that various factors other than bacterial infection may be responsible for changes in the composition of the secretion; on the other hand, infection may so damage the secreting tissue that abnormal secretion will be noted for some time after the numbers of bacteria have declined to a normal level, as in the case of the right front quarter of Heifer No. 7.

The possibility cannot be disregarded that there may be another biological agent concerned in addition to bacteria; where the bacteria alone are present, the composition of the secretion is scarcely influenced, but when the hypothetical second agent is present, definite changes occur. Although no information

has been obtained to date in these studies which casts any light upon the possible nature of this second agent, its presence would serve to explain such inconsistencies as have been mentioned in the preceding paragraph.

In the present studies no definite relation appeared to exist between yield and composition of milk. Some quarters showing slightly higher levels of catalase values also yielded significantly less milk than the corresponding quarter, while others failed to show any difference. This does not alter the fact that in the case of definitely abnormal quarters, both yield and composition of the milk are often markedly affected (1).

The tremendous fluctuations in bacteria counts, frequently of a regular rhythmic nature, are of interest. Had sampling been confined to the afternoon milkings, the extremely high counts from certain quarters would have been missed almost entirely.

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## THE INTERRELATION OF LIPIDS IN THE BLOOD OF NORMAL RABBITS<sup>1</sup>

BY ELDON M. BOYD<sup>2</sup>

### Abstract

A differential lipid analysis was performed by oxidative micromethods on heparinized plasma and red blood cells of 89 normal healthy rabbits and the normal means and range of blood lipid values established. Within this normal range, the amounts of all lipids in plasma were found to increase at the same relative rate, but a similar relation was found only for phospholipid and cholesterol in the red blood cells. Sex, body weight, blood hemoglobin and season were found to be without effect on the average lipid content of the blood of normal rabbits.

In a previous contribution to this Journal (5), a study was reported of the relation between the amounts of the various lipids in human blood to increasing values of total lipid. In that paper, reference to literature bearing upon the subject was made. Briefly, it was recorded that within the range of normal values the concentration of all lipids in the blood plasma of man tended to increase at about the same rate whenever an increase occurred in total lipid, but that either above or below the normal range the concentration of certain lipids increased or decreased more rapidly than that of others. There was less correlation in amounts of lipids in the red and white blood cells than in plasma.

An investigation was later made, with rabbits as experimental animals, to study the relation between the lipid content of blood and the immunity reactions developed during vaccination or infection. In rabbits vaccinated against *Streptococcus viridans* no significant variations were encountered in the phospholipid or free cholesterol contents of plasma (7). One of the difficulties encountered in the latter work and noted in the results summarized in Table I of that report (7), was the apparently marked variation in the phospholipid and free cholesterol contents of plasma in 28 normal rabbits used as controls.

Rabbits have been extensively used in research on the physiology of lipids, but a review of the literature revealed that extensive studies of variations in the normal amounts of lipids in blood of rabbits have been very few in number. Of the more recent reports, Starup (9) has given values for four lipids—total lipid, neutral fat, total cholesterol and phospholipid—in the blood of 54 normal rabbits. Boyd (4) summarized values for seven lipids in the plasma of 10 normal rabbits used as controls. The lipid content of the blood plasma of rabbits is considerably lower than that of dogs or of man but it is within the same range as that found in another herbivorous animal, the guinea pig (6). When compared with man or dogs, certain enigmatic variations are encountered in the lipid metabolism of rabbits. For example, it is difficult, if not

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impossible, to produce a post-prandial lipaemia in this species by feeding fat (1). These species variations must be borne in mind whenever rabbits, and probably other rodents, are used in the investigation of problems in the physiology or pathology of lipids.

The results reported in the present paper were anticipated to yield information on three points, first the range of blood lipid values in normal healthy rabbits, second the relation between the amounts of the various lipids and changes in the amount of total lipid, and third, an adjudication of the reputed importance of certain factors on the concentration of blood lipids. A total of 89 rabbits was used, 15 being females and the rest males. All of the animals appeared on examination to be healthy and most of them had been recently obtained from the farms. They varied in weight from 1,900 to 3,000 gm. They were housed in individual cages in well ventilated and uniformly heated quarters and were fed on a cereal mixture, containing the necessary foodstuffs and vitamins, supplemented by green vegetables from time to time.

Blood for lipid analysis was withdrawn without anaesthesia from the marginal ear veins of animals fasted over night and collected in flasks containing heparin as an anticoagulant. Extracts of plasma and of the red blood cells were made and analyzed by oxidative micromethods as used in previous studies.

### *Normal Values*

### **Results**

In Table I the lipid values obtained in the blood of this group of normal rabbits have been summarized. The significance of each value for each lipid in Table I and the derivation of the several values are the same as those given in Table III of the former paper on interrelations in man (5). The coefficient of variation listed in Table I is the standard deviation expressed as a percentage of the mean.

The blood *plasma* of normal rabbits was found to contain about one-half as much lipid per 100 cc. as the plasma of man. There was somewhat more than one-half as much neutral fat and less than one-half ester cholesterol and total cholesterol. The relative variations of lipid values of plasma were two to three times as great in rabbits as the corresponding variations in man. Reduced to units by comparing corresponding coefficients of variation, total lipid of rabbit plasma was 164% more variable in amount, neutral fat 14%, phospholipid 147%, free cholesterol 247% and ester cholesterol 126% more variable than in man (5). Undoubtedly a good deal of this variation is due to the smaller amounts of lipids in the plasma of rabbits. In other words, a variation of the same absolute amount of lipid would produce a greater change relatively in rabbit plasma than in human plasma. This partial interpretation is substantiated by the fact that the amount of neutral fat in the plasma of rabbits was nearer to that in human plasma than was the amount of any other lipid, and the coefficient of variation for the plasma neutral fat in rabbits was practically the same as that in man, this being the only coefficient not indicating considerably greater variation in rabbits than in man.

These results indicate that in comparing the effects of any experimental manipulation on the plasma lipid content of one group of rabbits with that of another group, a difference of practically 100% or more will be necessary before significance can be claimed for the difference. A change of this magnitude would probably always be pathological, so that the rabbit is not a very suitable animal for purposes of demonstrating lesser physiological differences between groups. This obvious restriction of the use of rabbits in research on the physiology of lipids is not meant to apply to repeated observations on the same animal. The significance of results from the latter type of experiment would depend upon other factors.

The lipid content of the *red blood cells* of normal rabbits (Table I) was found to be slightly lower on the average but not significantly lower than that of human erythrocytes (5). It is of interest that while the lipid content of plasma may differ greatly, that of the red blood cells remains almost identical in rabbits, man (5), guinea pigs (6) and dogs (2). Relative variation in the lipid content of the red blood cells was found practically the same in normal rabbits as in normal man.

TABLE I

THE LIPID CONTENT OF BLOOD IN NORMAL RABBITS

(Results expressed in mg. per 100 cc.)

Value	Total lipid	Composition of total lipid					
		Neutral fat	Total fatty acids	Cholesterol			Phospholipid
				Total	Ester	Free	
<i>Plasma—89 samples</i>							
Minimum	81	0	42	13	0	4	17
Maximum	482	231	311	100	54	59	181
Mean	243	105	169	45	23	22	78
Standard deviation	89	50	66	18	12	13	33
Coefficient of variation	37	48	39	40	52	59	42
Range of $\frac{1}{2}$ of values	154-332	55-155	103-235	27-63	11-35	9-35	45-111
Range of 95% of values	65-421	5-205	37-301	9-81	0-47	0-48	12-144
<i>Red blood cells—67 samples</i>							
Minimum	317	0	140	74	0	74	180
Maximum	646	211	411	150	36	136	358
Mean	433	63	251	112	5	107	259
Standard deviation	66	56	57	16	9	15	35
Coefficient of variation	15	89	23	14	180	14	13
Range of $\frac{1}{2}$ of values	367-499	7-119	194-308	96-128	0-14	92-122	224-294
Range of 95% of values	301-565	0-175	137-365	80-144	0-23	77-137	189-329

### Interrelation of Normal Values

Results in the 89 complete analyses of *plasma* summarized in Table I were divided into 10 groups containing four to fourteen complete analyses each, and arranged according to the value of the total lipid content. Group 1, for example, contained five complete analyses in which the total lipid content was below 125 mg. per cent; Group 2 contained seven complete analyses with total lipid values between 125 and 150 mg. per cent, and so on. The mean value of each lipid in each of these groups was then calculated and the means of the component lipids compared with the means for total lipid. As previously shown for human plasma (5), and as also found herein, there is not a significant difference between results in any two adjacent groups. But the coefficient of correlation between any lipid of plasma and the total lipid values shows that a significant correlation does exist between increasing values for the component lipid and those of total lipid.

The means of the component lipids have been plotted against the means of total lipid of plasma in Fig. 1. The abscissa of Fig. 1 is scaled arithmetically and represents the total lipid values. The ordinate is graduated in logarithms of the base 10 and on it the component lipid values are placed. Using an arithlog ruling in this manner permitted a more immediate visualization of the comparative relative changes in component lipid values with change in total lipid. It is obvious from Fig. 1 that with increase in total lipid of these *normal* rabbits, practically the same relative increase occurred in the amount of all of the component lipids. This substantiates the conclusion drawn from studies in human blood in which also about the same relative increases in the amount of component lipids occurred with increase in total lipid within the *normal* range (5).

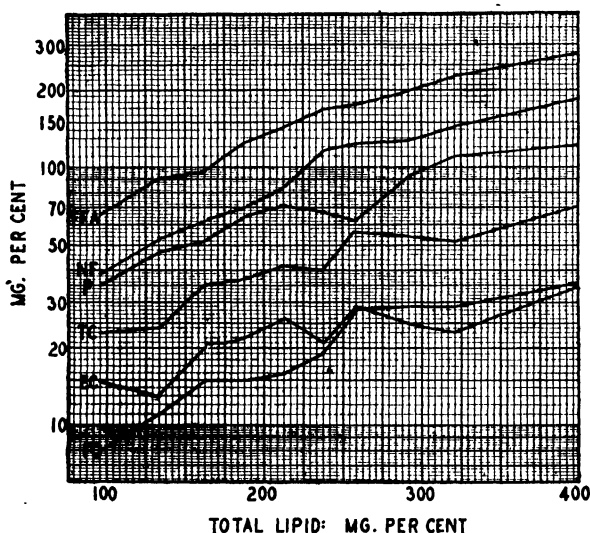


FIG. 1. Arithlog plotting of changes in the mean values of lipids of blood plasma of normal rabbits with increase in the total lipid. TFA = total fatty acids; NF = neutral fat; TC = total cholesterol; EC = ester cholesterol; FC = free cholesterol; P = phospholipid.

The lipids of the *red blood cells* showed less correlation when similarly arranged and plotted in Fig. 2. The amount of phospholipid, total cholesterol and free cholesterol increased at about the same proportional rate with increase in total lipid. The amount of total fatty acid increased at a somewhat faster rate owing to a fairly considerable increase in the mean value of neutral fat. There is relatively little neutral fat in most samples of red blood cells and the amount is extremely variable, so that actually there was not a significant increase in the amount of neutral fat with increase in total lipid. Fig. 2 demonstrates clearly the variable nature of the concentration of neutral fat in the red cells, and also of ester cholesterol, of which there is usually very little and most often none at all. It may be concluded that increase in the total lipid of the red blood cells of normal rabbits is accompanied by proportionate increases in phospholipid and cholesterol and occasionally by a marked increase in neutral fat with very little cholesterol ester present at any time. These relationships are identical with those described previously in human red blood cells (5).

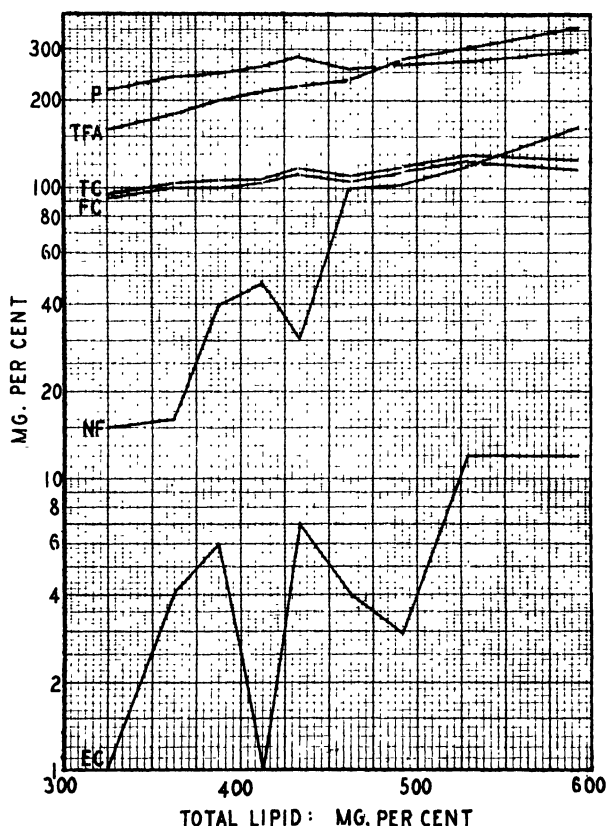


FIG. 2. Arithlog plotting of changes in the mean values of lipids of the red blood cells of normal rabbits with increase in total lipid. TFA = total fatty acids; NF = neutral fat; TC = total cholesterol; EC = ester cholesterol; FC = free cholesterol; P = phospholipid.

### *Certain Factors Supposed to Affect Blood Lipid Values*

**Sex.** The results were grouped according to the sex of the animal and a statistical analysis made of the two groups. There were found no significant differences in the lipid values between the two sexes. On the average, female rabbits contained 22% more phospholipid, 24% more ester cholesterol and 29% more free cholesterol in plasma than did the males. These average differences were not sufficiently great to be statistically significant.

**Body weight.** The analyses were divided into four groups according to the body weight of the animal, each group having a weight range of 300 gm. Again no statistically significant differences could be ascribed to variations in body weight. The plasma of the lighter and heavier animals contained the least average amounts of lipids. One group of middle-weight animals contained as much as 66% more phospholipid in plasma, but even this difference was insufficient to be significant. A similar difference found in man would likely be significant but the variations were so great in these rabbits that no real difference could be concluded to exist.

**Blood hemoglobin.** It is a well known fact that anemia is associated with changes in the lipid content of blood in man and in most animals including rabbits (1), although opposite variations are not seen in the antithetical condition of polycythemia (3). The hemoglobin content of the blood of these normal rabbits varied from 63 to 80% (Sahli) and it appeared possible that variation in hemoglobin concentration within the normal range might account for some differences in blood lipid values. Such was not found to be the case, however, when the analyses were grouped according to the hemoglobin value of the blood of the animal. No significant differences were found between lipid values in the several hemoglobin groups.

**Seasonal variation.** Blood was analyzed in a group of 12 rabbits in the winter, again in the spring and finally in the summer. The highest average values for plasma lipids were found in the spring and the lowest in the winter, but there was at no time a difference great enough to be significant. Nor was there any consistent increase or decrease in the lipid content of blood in individual animals from season to season. The logical conclusion was that changes in season had no consistent effect on the average blood lipid values of normal rabbits. McEachern and Gilmour (8) found no change in the average value of blood cholesterol and change in season in man.

### **Conclusion**

In conclusion, answers were obtained to the questions initially investigated. The lipid content of blood plasma and of the red blood cells of a large group of normal healthy rabbits was determined, the plasma values found to be about one-half as great as those in man and two to three times as variable, and the amounts of lipids in the red blood cells were shown to be similar in all respects to those in the red blood cells of man. Secondly, the same relative increase in the amount of all lipids of plasma was found to occur with increase in total lipid of plasma in normal rabbits, and like results were obtained in the red

blood cells, except that the increases in neutral fat and cholesterol esters were erratic, both relationships being analogous to those seen in normal human subjects. Thirdly, no relation could be established between blood lipid values in normal rabbits and variations in body weight, or the concentration of blood hemoglobin, or the season of the year, or between the two sexes.

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## ANALYSIS OF FLUCTUATIONS IN THE ACTIVITY OF INSECTS. A STUDY ON THE EUROPEAN CORN BORER, *PYRAUSTA NUBILALIS* HUBN.<sup>1</sup>

BY GEOFFREY BEALL<sup>2</sup>

### Abstract

The numbers of moths in flight, from night to night, over areas on which observations were made concurrently, show high correlations, although catch on non-contiguous areas differed more than would have been expected by chance. Polynomials of the fourth degree were found the most satisfactory relations to fit in determining seasonal trend of flight for each year.

The relations between numbers of moths in flight and physical conditions in the evening were investigated by the variate-difference procedure, using first differences. The numbers of moths and evening temperature showed a consistent moderate positive correlation. The numbers of moths and daily maximum temperature were positively correlated but the partial correlation, holding evening temperature constant, showed no relationship. The numbers of moths and evening humidity were not correlated, either simply, or as a partial correlation holding evening temperature constant. The numbers of moths and evening wind velocity, both simply and as a partial correlation holding evening temperature constant, showed a consistent moderate negative correlation.

For the relation between temperature and activity two successive approximations to a polynomial of the third degree were fitted jointly to all years. For temperatures below 60° F. there was no appreciable activity on the part of the moths. Activity increased with rise in temperature, although less rapidly as temperature became high. In general, activity at a given temperature was greater as the temperature of the preceding day was low.

### General Problem

It is generally accepted that environmental conditions affect the activity of insects. Many observations on the activity and rate of development of insects, under controlled physical conditions, show these phenomena to vary particularly with temperature.

In order to determine the nature of the relations between activity and physical conditions, entomologists have recorded activity in the field and have made observations on meteorological conditions, concurrently. In such work, it is desired to know what activity would have occurred at any given time under conditions other than those observed, or in places other than those where observations had been made. It is desired to evaluate times and localities with respect to their suitability for insect activity. Such a study has been made for some years at the Chatham laboratory on the European corn borer, *Pyrausta nubilalis* Hubn.

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In this paper the relation between activity of *Pyrausta nubilalis* Hubn. and physical conditions is examined. The methods of examination may be of use for insects in general. The sampling error of such types of observations is considered. The best means for estimating activity from a knowledge of physical conditions is sought.

### Historical Summary of Work on Problem

A short summary of efforts to find the relation between activity and physical conditions will give an idea of the standing of the problem. It may be noted that problems on rate of development are generally considered very similar to problems on degree of activity. In much of this work some preconceived relationship is fitted to data by subjective methods.

McEwan and Michael (7) use a method of solving for the relation between rate of biological processes and meteorological conditions. These workers break the series of values of one meteorological condition into groups, and then find the average value of the biological process rate for each group to obtain a first approximation to a relationship. The effect of the first meteorological condition is eliminated on the basis of this relationship and a first approximation to the partial relationship for the second obtained. Successive approximations back and forth between the two conditions are made. Cook (4) studies the relation between numbers of moths flying into bait traps and temperature. He correlates deviations from a seasonal trend with temperature, but determines no regression. The seasonal trend is found by the use of a sliding average. Glenn (5), in a study on rate of codling moth development, assumes a  $\wedge$ -shaped relationship, between rate of development and temperature, to exist. He tries various values for the feet and peak until he gets what he considers to be the best relationship. In studies on development under controlled physical conditions, Shelford (9) fits, by eye, a Krogh curve for rate of development against temperature. Williams and Bishara (15), in a study on activity of butterflies, take the average flight for each day of a particular temperature as the number of insects to be expected at that temperature. A comparison between the numbers at different temperatures gives the relative activity for the two temperatures. Janisch (6) works on the relation of activity to temperature and upon the effect of a given temperature upon subsequent activity. He fits curves upon the basis of the temperature at which activity is maximal.

### Data Employed

Data on the numbers of moths of *Pyrausta nubilalis* Hubn., taken at Chatham, Ontario, are considered in this paper. The number given for each evening is that observed in a plot of field corn during the years 1927 to 1933, inclusive. These observations and also meteorological readings were made at intervals of an hour during each evening. In 1927, moth flight was recorded in four sub-plots, and in 1929, flight was recorded in a field adjacent to the laboratory plot. All these data are presented in Table I. It may be noted

that in 1927 the collections made before July 11 are not comparable to those made later in the season and that the values for 1928 were doubled in a previous publication (13) for reasons stated therein.

TABLE I  
NUMBER OF MOTHS OBSERVED IN FLIGHT DURING ENTIRE EVENING

Date	1927					1928	1929		1930	1931	1932	1933
	Sub-plot 1	Sub-plot 2	Sub-plot 3	Sub-plot 4	Total		Laboratory plot	Secondary plot				
June 22												—
23												0
24												10
25												3
26									—			15
27									0		—	30
28									0		3	6
29									1	0	8	36
30									14	1	1	33
July 1									0	1	0	14
2							—	—	0	1	0	0
3							0	0	0	0	0	14
4							1	0	8	6	0	22
5							4	4	10	4	11	28
6						—	0	2	3	3	16	29
7	—	—	—	—	—	1	14	22	5	2	1	22
8	1	1	0	0	2	0	8	21	55	5	21	9
9	0	2	0	2	4	6	6	24	24	9	16	7
10	14	3	5	4	26	0	0	1	25	0	20	4
11	4	3	5	12	24	5	1	7	3	2	12	3
12	8	9	26	30	73	5	12	24	4	7	19	12
13	18	25	17	56	116	0	3	24	1	12	8	6
14	11	16	29	41	97	9	1	3	0	4	9	8
15	12	17	12	12	53	4	4	12	1	6	2	4
16	15	23	20	34	92	32	5	10	6	3	9	2
17	9	15	13	13	50	12	14	43	7	0	7	1
18	18	2	7	26	53	18	0	0	0	3	5	3
19	0	0	0	0	0	12	0	0	0	3	6	2
20	2	3	5	6	16	12	0	1	1	0	2	1
21	8	25	23	34	90	14	3	6	0	0	0	0
22	2	0	2	11	15	17	6	12	0	0	3	1
23	2	3	9	16	30	4	6	11	0	0	0	0
24	2	3	3	13	21	8	2	2	1	0	2	0
25	5	3	8	7	23	3	3	6	0	0	0	0
26	2	3	3	11	19	2	3	2	0	0	0	—
27	1	4	5	11	21	7	4	3	0	0	0	0
28	1	2	2	5	10	0	2	6	—	0	0	0
29	0	0	0	0	0	0	3	1		0	0	0
30	1	5	2	8	16	2	2	1		0	—	
31	1	0	1	3	5	3	1	0		—		
Aug. 1	0	0	0	0	0	0	0	0				
2	0	0	0	0	0	3	0	—				
3	0	0	0	3	3	3	0					
4	0	0	0	0	0	0	0					
5	0	0	0	2	2	0	0					
6	0	2	1	1	4	0	0					
7	1	1	0	0	2	1	0					
8	0	0	0	0	0	0	0					
9	—	—	—	—	—	2	—					
10						0						
11						—						





The physical conditions half an hour after sunset are considered typical of an evening because the main flight of corn borers occurs shortly after sunset. In Table II, evening temperature, in degrees Fahrenheit, evening relative humidity, as a percentage, evening wind velocity, in units of ten feet per minute, and also daily maximum temperature, in degrees Fahrenheit, are shown. General discussion on the method of observation has been published (12, 13).

### Notes on the Life History of the European Corn Borer

*Pyrausta nubilalis* Hubn. hibernates as a mature larva and in the late spring goes into the pupal stage. Spencer (10), working on females of the Ontario race of corn borers in 1921 and 1922, found longevity of moths to be between 15 and 17 days and to range from 7 to 31 days. In this material the pre-oviposition period averaged slightly less than 4 days and ranged from 2 to 9 days. Working on the same race, Poos (8) found the duration of each stage to be about as long and even more variable. The latter worker also found emergence from the pupal stage to continue all through July. It can be seen that among corn borers where all members of a group were held under the same conditions, by these workers, there was great variability in the duration of the stages. Caffrey (2) found that moths may fly five miles; therefore dispersion must tend to equalize local fluctuations in the rate of development and in moth population, over a wide area, and must further spread the occurrence of corn borer moths in a given field.

### Chance Error of Observations

It would be of use to see what magnitude of variation can be expected by chance in observations of  $n_s$ , the total number of moths seen in flight on the  $s^{\text{th}}$  evening. Let the  $s^{\text{th}}$  evening be any evening during the periods for which data were collected. Difficulty is encountered in estimating the magnitude of chance variations in observations of  $n_s$  because the observation of each evening is an isolated value and it is impossible, strictly, to repeat it. However, parallel observations were made in 1927 on four sub-plots and in 1929 on two plots. An examination is first made to determine to what extent catches made at one time on different areas were alike. Next, an examination is made to ascertain whether the catches on various sub-plots can be supposed to differ as they did, by chance. Finally, the results from this work are examined to determine in what way the magnitude of chance variation is related to the observed numbers.

To determine the similarity of the catch on the various sub-plots, the correlation between plots, for the numbers of moths taken each evening, is calculated. The data used run from July 8 to August 7, inclusive. The correlation coefficients between the data from the various pairs of sub-plots are as shown in Table III.

The correlation between catches from adjacent plots from which collections were made at one time is further investigated in the case of the two plots

TABLE III

CORRELATION BETWEEN CATCHES FROM DIFFERENT  
SUB-PLOTS OF 1927

—	Sub-plot II	Sub-plot III	Sub-plot IV
Sub-plot I	+ .72	+ .70	+ .66
Sub-plot II		+ .84	+ .83
Sub-plot III			+ .86

of 1929. The work resembled that done on the data from the sub-plots of 1927. The correlation between the catches from the laboratory plot and a secondary adjacent plot, in 1929, is calculated. The data used run from July 4 to July 31, inclusive. The correlation coefficient is +.85.

The correlation between catches on the sub-plots of 1927 and on the two plots of 1929 is high. This result shows that the numbers of moths caught on any one area vary with the numbers caught concurrently on other areas. Therefore, the nightly fluctuations in the number of moths from the various areas were not peculiar to the individual areas. Apparently, whatever controlled flight over one area did the same over the other areas.

While the catch from the various sub-plots of 1927 and from the two plots of 1929 may be highly correlated, the correlation may be largely due to seasonal trend. In examining the data from the four sub-plots in 1927, the question at once arises of how far the number of moths in each agrees with that in the others, because the numbers fluctuate from night to night about the seasonal trend. It is obvious that the number of moths might increase or decrease in all the sub-plots at about the same time of year, yet the nightly deviations from this trend might be largely independent. Accordingly, parallel observations between the four sub-plots of 1927 are examined to see with what degree of probability the sub-plots can be considered parts of one sample. The method of attack is to suppose the flight in the four sub-plots to be essentially identical and then to consider the probability that the catch would have differed, by chance, as much as was observed. The four sub-plots are considered in pairs to find how far flight on each pair might be considered identical. The value of  $\chi^2$  for each pair of sub-plots is calculated.

The data are grouped for the evenings July 8 to 10, inclusive, for July 18 and 19, for July 20 and 21, for July 22 and 23, for July 24 and 25, and for the evenings July 26 to August 7, inclusive. Thus, for each sub-plot, thirteen values of reasonable magnitude are available. The results are as shown in Table IV.

Since with twelve degrees of freedom the 0.05 and 0.01 levels of probability for  $\chi^2$  are 24.054 and 26.217, respectively, it is apparent that only in two cases are non-significant values of  $\chi^2$  found. It is ap-

TABLE IV  
VALUES OF  $\chi^2$  FOR CATCH FROM  
VARIOUS SUB-PLOTS OF 1927

—	$\chi^2$
Sub-plots I, II	32.915
Sub-plots I, II <sup>1</sup>	34.450
Sub-plots I, IV	44.327
Sub-plots II, III	22.451
Sub-plots II, IV	40.593
Sub-plots III, IV	22.050

parent that the catch from the pairs of contiguous plots, II and III, and III and IV, only, differed as little as can be expected by chance. The most widely removed plots, I and IV, differed to the greatest extent. Apparently there was some systematic variation in catch over the plot as a whole.

In order to see how the numbers of moths coming to a given plot should vary by chance, suppose one had had an infinite number of like plots to which moths might fly randomly. On the  $s^{\text{th}}$  evening a moth might go to any one of a large number of plots similar to that being used for observations. The probability of it coming to the laboratory plot would have been, therefore, very small. Since, however, many moths were available in a district, the number actually coming to any plot would have been moderately great. If the total number of moths coming to the given plot on the  $s^{\text{th}}$  evening were  $n_s$ , the mean number coming to all the plots would have been  $\bar{n}_s$ . The values of  $n_s$  would have varied about  $\bar{n}_s$  in a Poisson distribution.

As a study of whether the magnitude of the deviation of  $n_s$  really fell anywhere near  $\sqrt{\bar{n}_s}$ , the catches on the sub-plots of 1927 are supposed to be all part of one flight and the deviations of the various sub-plots are supposed to be due to chance. The catches have been found, above, to fluctuate together, although the discrepancies were a little greater than might be expected by chance. It is supposed that from the four sub-plots one can get for any one sub-plot an expectation of flight. This expectation is found in the same way that expectation is calculated in making the  $\chi^2$  test. Let  $n_{si}$  be the catch on the  $s^{\text{th}}$  night in the  $i^{\text{th}}$  plot, and  $\epsilon_{si}$  be the corresponding expectation. Deviation of  $n_{si}$  from  $\epsilon_{si}$  should vary with, approximately,  $\sqrt{\epsilon_{si}}$  as a standard deviation. Accordingly, the values of  $\frac{n_{si} - \epsilon_{si}}{\sqrt{\epsilon_{si}}}$  should be approximately distributed with standard deviation of unity.

Such values  $\frac{n_{si} - \epsilon_{si}}{\sqrt{\epsilon_{si}}}$  are calculated for the data of the sub-plots of 1927. These values, called  $X$ , are plotted in Fig. 1 against the total number of moths, called  $Y$ , in the four sub-plots on the  $s^{\text{th}}$  evening. The data used run from July 8 to August 11, inclusive. In order to secure entries of satisfactory magnitude, observations are grouped for the evenings July 8 to 10, inclusive, for July 18 and 19, for July 28 to 30, inclusive, and for July 31 to August 11, inclusive. The ratio  $\frac{n_{si} - \epsilon_{si}}{\sqrt{\epsilon_{si}}}$  is independent of meteorological conditions, or of seasonal trend on the various nights, since it is only concerned with the fluctuations on a single night.

In Fig. 1, it can be seen that of the 76 values of  $\frac{n_{si} - \epsilon_{si}}{\sqrt{\epsilon_{si}}}$ , 7 fall beyond  $\pm 2$ , 22 fall beyond  $\pm 1$ , and 47 fall within  $\pm 1$ . The corresponding figures from the normal curve would be 3.5, 20.7, and 51.9. Accordingly, the observed values are somewhat of the magnitude to be expected. On the whole, the absolute magnitude of values of  $X$  appears to be independent of the magnitude of  $Y$ , although, possibly,  $X$  increases slightly with  $Y$ .

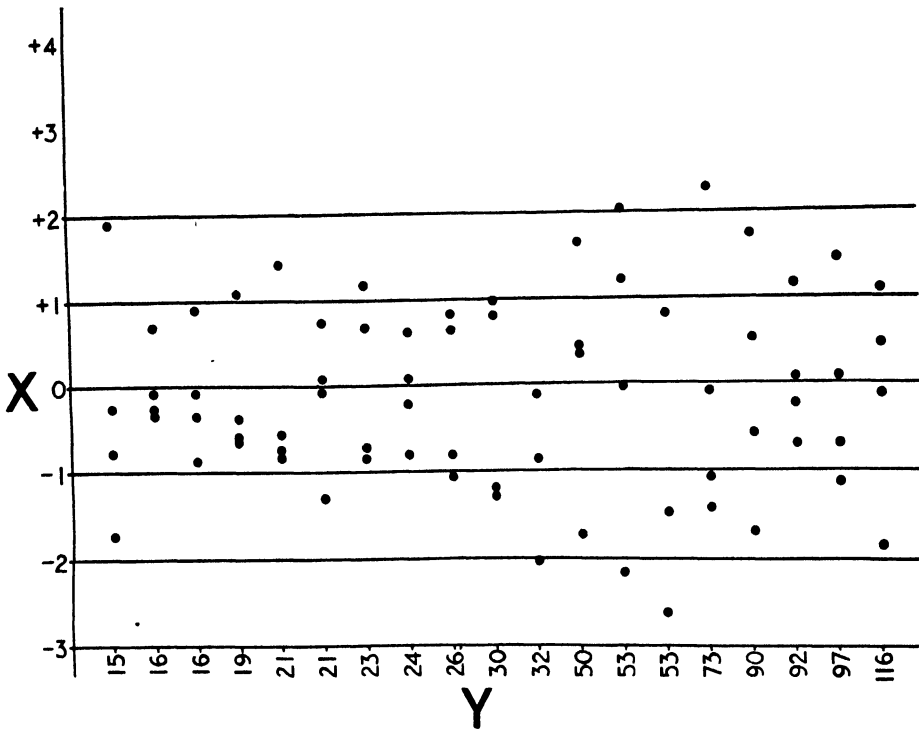


FIG. 1. Deviations of sub-plots of 1927 from plot expectation in terms of expected standard deviation.

The data from the four sub-plots, from which collections were made concurrently, support the hypothesis that if instead of any one value of  $n_s$  one had a series, they would tend to fluctuate about  $\bar{n}_s$  with a standard deviation of  $\sqrt{\bar{n}_s}$ . This finding suggests an estimate of variation for the value of flight as noted each evening. Using this estimate one can say whether the flight on any two successive evenings is significantly different.

### Estimation of One Quantity from Observation of Another

Ability to estimate fluctuations in one quantity from a knowledge of fluctuations in another would be extremely useful in field work. For instance, if one knew the fluctuations from night to night in the catch in a light trap he might estimate the corresponding fluctuations in flight in a field of corn. Again, if one knew the latter fluctuations he might estimate fluctuations in rate of oviposition. From an understanding of the effect of meteorological conditions and of seasonal trend upon numbers of moths seen, one could estimate  $n_s$  from an observation of  $n_{s-1}$ .

Suppose one had observations on flight of moths over one plot and would estimate simultaneous flight, from evening to evening, over another plot. One supposes that the difference in fluctuation, from evening to evening, in observations over the two areas are mainly due to chance variations in the

numbers observed. One wishes to estimate the flight on the  $s^{\text{th}}$  evening in Plot II from the flight in the same evening on Plot I, if it is known that the flight on Plot II is  $\lambda$  times as great as that on Plot I. Call the flight on Plot I,  $n_{s1}$ , and the flight on Plot II,  $n_{s2}$ . Since one does not know  $\tilde{n}_{s1}$  for the first plot, that is what the mean catch would have been if the observations could have been repeated indefinitely, but only  $n_{s1}$ , one must estimate on the basis of the latter quantity. On the basis of  $n_{s1}$  make an estimate of  $n_{s2}$ , namely  $\tilde{n}_{s2}$ . As discussed above, chance variations in  $n_{s1}$  appear to form a Poisson distribution and to have a standard deviation of approximately  $\sqrt{n_{s1}}$ . If one may expect  $n_{s2}$  to be  $\lambda$  times greater than  $n_{s1}$ , the distribution of  $n_{s1}$  is stretched  $\lambda$  times to form the distribution of  $\tilde{n}_{s2}$ , and the latter quantity would be calculated with a standard deviation of approximately  $\lambda\sqrt{n_{s1}}$ . The observation of  $n_{s2}$  may also be supposed to vary in a Poisson distribution with a standard error of approximately  $\sqrt{n_{s2}}$ . Therefore, the difference between  $n_{s2}$  and  $\tilde{n}_{s2}$  should be approximately distributed with a standard deviation of  $\sqrt{n_{s2} + \lambda^2 n_{s1}}$ .

As an example of estimation of observations over one plot from those on another, estimates of flight over the laboratory plot in 1929 are made from the numbers of moths observed, concurrently, over the secondary plot of the same year. The estimates are compared with the observation actually made.

The laboratory plot is referred to as Plot II, and the secondary plot is referred to as Plot I. The catches from these two plots were shown, above, to be highly correlated. Since the total catch on laboratory plot is 108 and on the secondary plot is 248,  $\lambda = \frac{108}{248}$ .  $\lambda$  is used to calculate  $\tilde{n}_{s2}$  from  $n_{s1}$ . A series of differences,  $(n_{s2} - \tilde{n}_{s2})$ , is obtained. These quantities

TABLE V

COMPARISON OF OBSERVATIONS OVER ONE PLOT WITH THE ESTIMATES OF THESE OBSERVATIONS

Date	$n_{s1}$	$\lambda n_{s1} = \tilde{n}_{s2}$	$n_{s2}$	$n_{s2} - \tilde{n}_{s2}$	$\sqrt{\lambda^2 n_{s1} + n_{s2}}$	$\frac{n_{s2} - \tilde{n}_{s2}}{\sqrt{\lambda^2 n_{s1} + n_{s2}}}$
July 4-6	6	2.6	5	+2.4	2.48	+1.0
July 7	22	9.6	14	+4.4	4.26	+1.0
July 8	21	9.1	8	-1.1	3.46	-0.3
July 9-11	32	13.9	7	-6.9	3.62	-1.9
July 12	24	10.5	12	+1.5	4.07	+0.4
July 13	24	10.5	3	-7.5	2.75	-2.7
July 14-15	15	6.5	5	-1.5	2.80	-0.5
July 16	10	4.4	5	+0.6	2.63	+0.2
July 17	43	18.7	14	-4.7	4.71	-1.0
July 18-21	7	3.0	3	0.0	2.08	0.0
July 22	12	5.2	6	+0.8	2.88	+0.3
July 23	11	4.8	6	+1.2	2.84	+0.4
July 24-25	8	3.5	5	+1.5	2.55	+0.6
July 26-27	5	2.2	7	+4.8	2.82	+1.7
July 29-31	8	3.5	8	+4.5	3.09	+1.5
	248		108			

should be approximately normally distributed with a standard deviation of, approximately,  $\sqrt{n_{s2} + \lambda^2 n_{s1}}$ . Since the catch on some days was very small, certain days were grouped. On this basis one obtains the series of calculations shown in Table V.

It can be seen that of the fifteen values of  $\frac{n_{s2} - \tilde{n}_{s2}}{\sqrt{\lambda^2 n_{s1} + n_{s2}}}$ , one falls beyond  $\pm 2$ , 4.5 fall beyond  $\pm 1$ , and 9.5 fall within  $\pm 1$ . The corresponding figures from the normal curve would be 0.7, 4.1, and 10.2. Accordingly, it appears that the differences between  $n_{s2}$  and  $\tilde{n}_{s2}$  are of about the magnitude to be expected by chance. The high degree of inaccuracy of the estimate of the  $n_{s2}$ ,  $\tilde{n}_{s2}$ , made upon  $n_{s1}$ , is obvious. Since the accuracy of  $\tilde{n}_{s2}$  varies with the magnitude of  $n_{s1}$ , upon which it is based, far greater values of  $n_{s1}$  than those observed would be necessary to make such a method of much use.

### Investigation of the Relation Between Numbers of Moths in Flight and Meteorological Conditions by the Variate-difference Method

As discussed elsewhere in this paper, the effect of seasonal trend upon numbers of moths in flight is marked and must be eliminated before the relation between these numbers and meteorological conditions can be determined. In order to eliminate the effect of seasonal trend from observations on numbers of moths in flight one may use the variate-difference method of correlation (3). This method was, of course, developed to eliminate just such influence of trend. While the variate-difference method furnishes a quantitative estimate of the relation between activity and meteorological conditions, it does not show the general nature of the relationship. The method is used in this paper to find whether there is a relation between number of moths in flight and evening temperature, daily maximum temperature, relative humidity, or wind velocity. Differences in physical conditions are examined against differences in catch for *Pyrausta nubilalis* Hubn.

The effect of evening temperature, which may be expected to be the most important meteorological condition affecting flight of moths, is first examined by the variate-difference method. For temperature of the evening, temperature at 30 minutes after sunset is used. An examination of the values obtained by correlating various differences of temperature and numbers of moths in flight is made with the purpose of finding what difference is the most satisfactory. The results obtained in this work are shown in Table VI.

TABLE VI  
CORRELATION OF SUCCESSIVE DIFFERENCES OF NUMBERS OF MOTHS AND OF EVENING TEMPERATURE

Year	Date range, inclusive, of data	Raw numbers $r_{tn}$	First difference $r_{tn}$	Second difference $r_{tn}$	Third difference $r_{tn}$
1928	July 7 - Aug. 3	+ .23	+ .23	+ .18	+ .15
1929	July 5 - July 29	+ .36	+ .58	+ .56	+ .53
1930	June 30 - July 20	+ .15	+ .26	+ .29	+ .37
1931	July 3 - July 21	+ .06	+ .45	+ .45	+ .43
1932	June 29 - July 24	+ .18	+ .44	+ .53	+ .59
1933	June 24 - July 23	+ .50	+ .52	+ .46	+ .44

In general, the first difference showed a higher correlation than further differences. Accordingly, it is assumed that correlation of first differences is probably the most reasonable procedure. In the examination of the relation between numbers of moths and physical conditions, other than temperature, first differences are studied. One finds that values of  $(n_s - n_{s-1})$  at the beginning and end of the season of flight, when seasonal trend may be supposed to be low, tend to be smaller than those obtained during the middle of the season. The smallness of these differences arises because the influence of seasonal trend has not been completely eliminated. Also, since it has been demonstrated that the magnitude of chance errors depends on the magnitude of flight and since the magnitude of flight depends, in part, on seasonal trend, then the magnitude of chance errors at the ends of the flight period must tend to be small. Accordingly, they must introduce chance variations of but small magnitude into differences of observations at these times. Further differences than the first are not profitable since the chance error of observations tends to be accentuated.

From Table VI it can be seen that in all years, and for all differences, the numbers of moths in flight and temperature are positively correlated. Therefore, it is certain that the number of moths taken increases with rise in temperature, in general.

The results for 1931 are of some interest. The correlation between temperature and numbers of moths, raw numbers, is very low, +0.06, and the correlation of first differences moderately high, +0.45. This result occurs because the temperatures at the end of the period of flight rose, as seasonal trend of flight fell. First differences, however, eliminated much of the effect of the trend.

*Pyrausta nubilalis* Hubn. flies in the evening when temperature is comparatively low. One may suppose, however, that the temperature during the warmer part of the day has an effect upon evening flight. Thus, the heat of the day may control the number of eggs matured and laid in the evening.

The relation between numbers of moths in flight and maximum temperature is examined in the same way as that between flight and evening temperature.

TABLE VII  
CORRELATION OF FIRST DIFFERENCES OF MOTH NUMBERS  
AND OF MAXIMUM TEMPERATURE

Year	Date range, inclusive, of data	$r_{nj}$	$r_{nj,t}$
1928	July 7 - Aug. 3	+ .28	+ .18
1929	July 4 - July 29	+ .47	+ .19
1930	July 1 - July 20	+ .05	- .06
1931	July 3 - July 19	.00	- .36
1932	June 29 - July 24	+ .25	- .22
1933	June 24 - July 23	+ .30	- .11

Since evening temperature and daily maximum temperature must be closely related, in addition to the correlation of number of moths in flight ( $n_s$ ) and maximum temperature ( $j_s$ ), a partial correlation is calculated, holding evening temperature ( $t_s$ ) constant. The results are shown in Table VII.

The results from this examination show that flight is correlated positively with daily maximum temperature. However, such a relation does not exist when the partial correlation, holding evening temperature constant, is calculated. In this case one may say that there is not a significant relation between activity and maximum temperature, or else that linear relation is not adequate.

Humidity might have been expected to have a very great effect upon activity. The relation between humidity and numbers of moths in flight is examined in the same way as that between flight and maximum temperature. In addition to the simple correlation of numbers of moths in flight ( $n_s$ ) and humidity ( $h_s$ ), a partial correlation is calculated holding evening temperature ( $t_s$ ) constant. Relative humidity at half an hour after sunset is used. The results are shown in Table VIII.

From the results of this examination one may conclude that humidity as measured, has, contrary to expectations, but little control over activity.

For wind ( $w_s$ ), as for daily maximum temperature, and for humidity in the evening, correlation of first difference with first difference of numbers of moths in flight, and the partial correlation holding evening temperature constant, are calculated. Wind velocity at half an hour after sunset is used. The results are shown in Table IX.

The results from this examination show that wind has a depressing effect on flight. Since the correlation between evening temperature and wind velocity is low, the partial correlation between wind and numbers of moths, holding temperature constant, shows a clear-cut, negative relationship.

The depressing effect of wind upon flight must be very strong, since one finds such a marked negative relation between wind velocity readings for one hour and numbers of moths in flight for an evening. Wind velocity values taken thirty minutes after sunset are probably not a good index of wind velocities for the entire evening, since wind velocity changes rapidly

TABLE VIII  
CORRELATION OF FIRST DIFFERENCES OF MOTH NUMBERS  
AND OF HUMIDITY

Year	Date range, inclusive, of data	$r_{hn}$	$r_{hn.t}$
1928	July 7 - Aug. 3	— .14	— .02
1929	July 5 - July 29	— .22	— .39
1930	June 30 - July 20	+ .04	+ .11
1931	July 3 - July 21	+ .43	+ .33
1932	June 29 - July 24	— .16	— .18
1933	June 24 - July 23	— .16	.00

TABLE IX  
CORRELATION OF FIRST DIFFERENCES OF MOTH NUMBERS  
AND WIND VELOCITY

Year	Date range, inclusive, of data*	$r_{wn}$	$r_{wn.t}$
1928	July 7 - Aug. 3	— .22	— .22
1929	July 5 - July 29	— .21	— .16
1930	June 30 - July 20	— .32	— .33
1931	July 3 - July 21	— .51	— .51
1932	June 29 - July 24	— .34	— .29
1933	June 24 - July 23	— .36	— .30

\*Less lacking observations, see Table II.

and erratically. No one value of wind velocity should be so typical of conditions for an evening as such a value of temperature. Temperature values taken soon after sunset should be a fairly good index of evening temperatures, since temperature changes comparatively slowly, and changes systematically.

One may sum up this examination of the relationship between numbers of moths and various physical factors by the variate-difference method, as follows. A powerful influence is exercised by evening temperature on numbers of moths, which increase with temperature. No clear-cut relation between activity and either maximum daily temperature or humidity is apparent. Wind velocity exercises a depressing effect upon flight.

### Definition of Activity

The remainder of this paper is mainly concerned with the relation between activity of the corn borer moths and temperature. This relationship is investigated at some length since, as was shown above, the number of moths in flight is closely associated with evening temperature. Accordingly, it is useful to determine the relationship more closely than has been done. Also the methods of investigation may be useful for further work in determining other relations.

Since in the remainder of this paper the discussion is of activity under given conditions of evening temperature, the term "activity" should be defined. The activity of the moths is thought of as the extent to which moths came to the observation plot, compared with the extent to which they would have come under average temperature conditions. Accordingly, we take activity to be the ratio of the mean number of corn borer moths to the mean number which would be seen under average conditions of evening temperature, if observations could be repeated indefinitely. More exactly, we may say  $\frac{\bar{n}_s}{D_s}$  is the activity,  $A_s$ , on the  $s^{\text{th}}$  evening. The value,  $\bar{n}_s$ , is as previously defined. The value,  $D_s$ , is the mean number of moths flying in a given observation plot under temperature conditions average for the period under examination, when an infinite number of such plots are observed.

In field work, as discussed in the introduction, one is mainly concerned with the relative activity obtaining between two given conditions. That is, one wishes to know how the catch under given conditions would compare with that under other conditions. The relative activity of the  $s^{\text{th}}$  and  $x^{\text{th}}$  evenings is  $\frac{A_s}{A_x}$ , or  $\frac{\bar{n}_s}{D_s} / \frac{\bar{n}_x}{D_x}$ . Such a value measures the relative suitability for flight of the  $s^{\text{th}}$  and of the  $x^{\text{th}}$  evenings.

### Choice of Appropriate Seasonal Trend Lines

In finding the relation between activity and temperature, or other physical conditions, it is first necessary in some way to estimate seasonal trend of flight. The relation must be found by relating estimates of activity for each evening to the temperature conditions prevailing on that evening. From the nature of activity, as defined above, some estimate of the number of moths

to be expected under average temperature conditions must be made. It is shown below that the number of moths to be expected each evening, under average temperature conditions, varies with date of the evening. Accordingly, the effect of seasonal trend of flight must be estimated.

The magnitude of flight to be expected under any given temperature conditions varies greatly with the progress of the season of flight, since the entire season of flight of *Pyrausta nubilalis* Hubn. lasts only about five weeks. When flight starts, few moths are taken; for a week or so the numbers taken increase rapidly; later, the numbers decrease. This general seasonal trend of flight has occurred in observations made during seven years, in several fields, as can be seen in Table I.

In addition to the general impression obtained from observations on seasonal trend of corn borer moth flight, something of the nature of the trend may be learned from the life history of *Pyrausta nubilalis* Hubn., previously discussed. It is apparent that the larvae emerge from the winter at practically one level of development. Within any group subject to the same conditions there is variation in the time taken to reach the imaginal stage and in the duration of the imaginal stage. Further, one may expect a continuous variation in the rates of development in different situations. Moths from all these situations must tend to come to a given field, since the moths travel widely and freely. Altogether, one may expect a continuous variation in the abundance of moths in a given field. One may expect the flight to commence each season gradually, reach a peak, and gradually die away, as appears to be roughly the case.

It is of interest to note that, when data from the various years are fitted with fourth degree polynomial curves, but one peak in flight is obtained, except for the data of 1931 which show two peaks. Even in this case the bimodality is a temperature effect.

In order to fit a line to seasonal trends, biological workers have made use of a sliding average. A mathematical trend is more desirable in that one can readily estimate the significance of deviations from it.

Polynomials have been used widely in various fields of work to fit for trend against time. In the final form of analysis of the relation between activity and temperature, polynomials are used in this paper. This is done since polynomials are comparatively easy to fit and the calculation of summed deviations, squared, of observations about them is simple. Also, the function of activity in terms of temperature is solved as a polynomial. Accordingly, the functions of temperature and of time are made analogous.

In addition to the use of polynomials in fitting to seasonal trend, the use of frequency curves was tried. It appeared that when one knows the flight to have the characteristics discussed above, one can apply less general curves than polynomials. It was thought frequency curves should be applicable since the flight must approach zero asymptotically at the ends of its duration, and is most probably unimodal. In particular, the use of curves of the Pearson, or of the Gram-Charlier systems, was examined. For the reasons indicated above, the polynomial was ultimately chosen.

## Estimation of Activity Relationships by Deviation from Trend Lines

It would, of course, appear that if one had fitted a line, even approaching the seasonal trend, one might take the deviations of observations from it as estimates of activity and relate these deviations to temperature. Such procedure was not, however, found feasible.

In the first place it should be pointed out that one does not want to fit for temperature conditions an equation of the same type and order as for date, and then try to relate the respective deviations. One wants a relation between activity and actual temperature conditions. If one knows such a relation he can apply the results in the field as soon as temperature observations are made. He can then estimate activity to be at a low, medium, or high level, according to physical conditions.

If one fit a seasonal trend line to observed data he does not obtain an estimate of the flight that would have occurred under average temperature conditions. He obtains, rather, a line taking into account only the values observed, and thus incorporating the effect of temperature. Thus, if for a period temperature was such as to depress or to inhibit flight, the trend goes down. However, during this period there might have been a great flight under average temperature conditions.

If one does fit a seasonal trend to the data of each year, and calls the trend value on the  $s^{\text{th}}$  night  $m_s$ , it would seem one might use, as the estimate of activity on the  $s^{\text{th}}$  night, the deviation of  $n_s$  from  $m_s$ . A good deal of work was done along this line. It was first thought to use  $\frac{n_s - m_s}{m_s}$  as an estimate of activity to find the relation of activity to temperature conditions. However, for this estimate of activity, random error varies approximately with  $\sqrt{n_s}$ . If temperature conditions are fairly suitable for flight,  $n_s$  tends to vary with the magnitude of flight possible, *i.e.*, with the time of year and with the year. Accordingly, chance error tends to vary with the time of season and magnitude of seasonal flight. In order to avoid this difficulty of variable random error, use of the quantity  $\frac{n_s - m_s}{\sqrt{m_s}}$  was made. In using this quantity one was faced with two difficulties. In the first place the random error does not vary as  $\sqrt{m_s}$  but as  $\sqrt{n_s}$ . In the second place, one had an estimate of activity dependent on the time of season in part, not solely on activity.

On account of the difficulty in fitting a trend that was independent of temperature, and of the difficulty of using deviations from a trend line, the methods discussed above are not used in this paper.

## Investigation by Comparing Successive Ratios for Flight with Changes in Temperature

Rather than attempt to estimate seasonal trend, and from it to attempt to estimate activity, one might choose an estimate of activity which is, to a certain extent, independent of trend. One can get such an estimate in the form of the quantity  $\frac{n_s}{n_{s-1}}$ , when studied in connection with  $t_s$  and with  $t_{s-1}$ . The

values,  $(s - 1)$  and  $s$ , are subscripts used to denote two successive evenings on which data were collected.

The value  $\frac{n_s}{n_{s-1}}$  furnishes an estimate of the relative activity between the temperatures  $t_s$  and  $t_{s-1}$ . The nature and value of estimates of relative activity are noted in the foregoing discussion on activity. Use of the quantity  $\frac{n_s}{n_{s-1}}$  is justified as follows. If, as was previously supposed, the activity,  $A_s$ , on the  $s^{\text{th}}$  evening with temperature,  $t_s$ , equals  $\frac{\bar{n}_s}{D_s}$ , then,  $\frac{n_s}{D_s}$  is an estimate of  $A_s$ . Similarly,  $\frac{n_{s-1}}{D_{s-1}}$  is an estimate of  $A_{s-1}$ . The difference between the values of seasonal trend on successive evenings should be comparatively small. If  $D_s$  differs but little from  $D_{s-1}$ , one obtains an approximation to  $\frac{A_s}{A_{s-1}}$  from  $\frac{n_s}{n_{s-1}}$ . Accordingly, in  $\frac{n_s}{n_{s-1}}$  one obtains an estimate of the relative activity associated with the temperature conditions,  $t_s$  and  $t_{s-1}$ .

In Table X, values of  $\frac{n_s}{n_{s-1}}$  are entered against  $t_s$  and  $t_{s-1}$ . In the compilation of this table, data on the flight of *Pyrausta nubilalis* Hubn. are used from the years 1928–1933, inclusive. Within these years the range of dates, inclusive, employed is as follows: in 1928 from July 7 to August 3, in 1929 from July 4 to July 30, in 1930 from June 29 to July 18, in 1931 from July 2 to July 19, in 1932 from June 28 to July 24, in 1933 from June 24 to July 22.

When both  $n_{s-1}$  and  $n_s$  are zero,  $\frac{n_s}{n_{s-1}}$  has, of course, an indeterminate value. Such values have been represented in Table X by the sign  $\sim$ . When  $n_{s-1}$  is zero, and  $n_s$  is greater than zero, the value  $\frac{n_s}{n_{s-1}}$  is, of course, infinite. Such values have been entered in the table as  $\infty$ . Other values of  $\frac{n_s}{n_{s-1}}$  have been entered as two-place decimals. A line has been drawn diagonally across the table through the region where  $t_s = t_{s-1}$ . Values to the left of this line, of course, occurred with falling temperatures and values to the right with rising temperatures. It may be pointed out that the quantities considered are total numbers of moths for each evening and temperature at thirty minutes after sunset.

From a table of such a quantity as  $\frac{n_s}{n_{s-1}}$  one cannot get a numerical estimate of the degree of association between activity and temperature. However, Table X does show the characteristics of the relation between flight and temperature.

If the values in Table X are examined with respect to their distribution about the diagonal, where  $t_s = t_{s-1}$ , two tendencies are discernible. First, observations below and to the right of the diagonal tend to be greater than 1.00 and those to the left to be less than 1.00. It is shown below that there

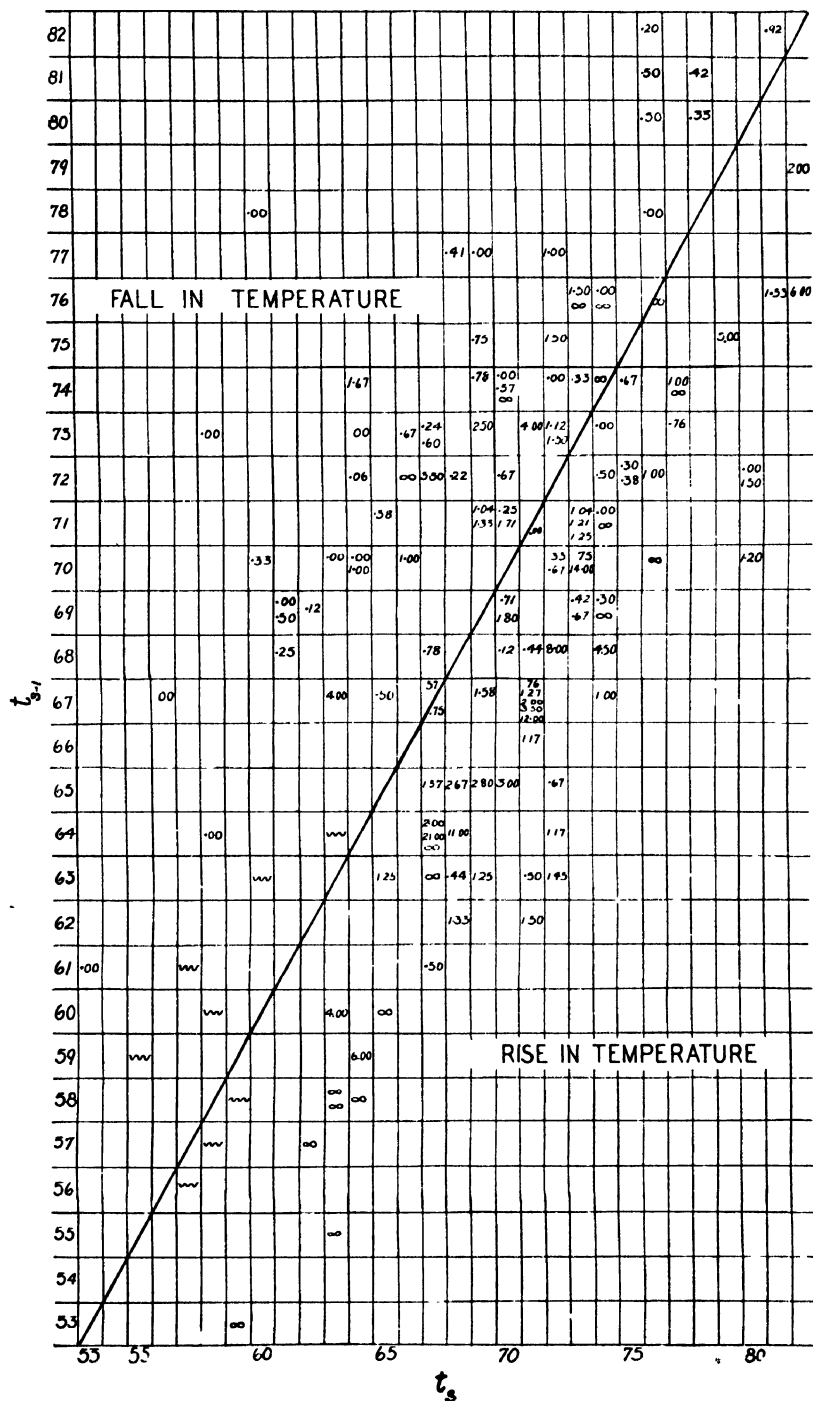
is a statistically significant tendency for values to be so arranged. Such a tendency means that relative activity falls when temperature falls and rises when temperature rises. Second, any two values of  $\frac{n_s}{n_{s-1}}$ , on any given perpendicular to the diagonal, and at equal distances from the diagonal, tend to be reciprocal. Throughout the field this tendency appears general. However, it is most intense in the region of the lower values of  $t_s$  and  $t_{s-1}$ . Such values of  $\frac{n_s}{n_{s-1}}$  should be so reciprocal if there are given levels of activity associated with given temperatures. Suppose activity  $A_1$  is associated with  $t_1$ , and activity  $A_2$  with  $t_2$ , where  $t_1 \neq t_2$ . Then, the two values of  $\frac{n_s}{n_{s-1}}$  are estimates of the values  $\frac{A_1}{A_2}$  and  $\frac{A_2}{A_1}$ , respectively.

The significance of the apparent tendency for relative activity to fall and to rise with temperature may be investigated by determining the probability that the values in Table X could have been distributed as they were by chance. Two groups of values, those greater than, and those less than 1.00 are considered. Values of 1.00 are put half in each of these classes. In the upper right-hand part of the field, where  $t_s$  and  $t_{s-1}$  are 60° F. or more, there are 126 entries. Of these, 56 fall in the region where  $t_{s-1}$  is greater than  $t_s$ , that is, where temperature had fallen; 5 fall in the region where  $t_{s-1}$  equals  $t_s$ ; and 65 fall in the region where  $t_{s-1}$  is less than  $t_s$ , that is where temperature had risen. Of the 56 in the region where  $t_{s-1}$  is greater than  $t_s$ ; 17.5 are greater than 1.00; 36.5 are less than 1.00. The two indeterminate values are not considered. If the size of the ratios had been determined by chance and a ratio had as much chance to be over 1.00, as under, then one would expect 27, with a standard deviation of 3.67, in each group. The deviation of 9.5 from 27 is therefore significant. Therefore, the number of values less than 1.00 is significant. That is, fall in temperature reduces flight. Of the 65 in the region where  $t_{s-1}$  is less than  $t_s$ , 41.5 are greater than 1.00 and 23.5 less than 1.00. If the size of the ratios had been determined by chance and a ratio had as much chance to be more than 1.00, as to be less than 1.00, then one would expect 32.5, with a standard deviation of 4.03, in each group. The deviation of 9.0 from 32.5 is, therefore, significant. Therefore, the number of values greater than 1.00 is significant. That is, rise in temperature increases flight.

From Table X it can be seen that, as was reported by Stirrett (12), for temperatures less than 60° F. there can be no appreciable activity. Activity at any temperature above 60° F. is, with one exception, infinitely greater than that observed at temperatures below 60° F. Also, when  $t_s$  and  $t_{s-1}$  are below 60° F., indeterminate values are obtained.

A surface fitted to data of the type shown in Table X would furnish a most useful relation between activity and temperature. An unsuccessful effort was made to fit such a surface. In the first place, a satisfactory surface was

TABLE X  
RATIO OF FLIGHT TO TEMPERATURE ON SUCCESSIVE EVENINGS



most difficult to find. In the second place, since the random error of the values  $\frac{n_s}{n_{s-1}}$  was not normal, and was very great, these quantities were very difficult to use. These values may vary between 0.00 and  $+\infty$  with half the values lying between 0.00 and  $+1.00$ . Some work on transformation of the values of  $\frac{n_s}{n_{s-1}}$  was attempted, but it was not satisfactory.

### Effect of Previous Temperature

Laboratory investigations by various workers show that rate of development of insects, at any given temperature, depends, in part, upon the temperature conditions to which the insect has been previously subject. Such work is reviewed by Uvarov (14). He says that "one point, however, is beyond dispute, namely, that fluctuations of temperature are not without an effect on the rate of development. This effect is often positive, particularly when a favorable temperature alternates with one below the zero of development (but not low enough to be injurious), while an alternation with high temperature is usually harmful." If one supposes rate of development and degree of activity to be similar phenomena, one would expect activity at a given temperature, when preceded by low temperatures to be different from, and probably greater than, that at the same given temperature preceded by high temperatures. However, little investigation seems to have been made of such possible modification of the relation between activity and current temperature. Accordingly, in this paper, an examination is made, below, of the possible modification.

In this paper an estimate of the relation between activity on a given night and temperature on that night and on the preceding night jointly is made. The solution must be joint, since the effect of previous temperature should not be shown directly but in the modification of the activity found with current temperature.

In the joint solution discussed above, only temperatures  $t_s$  and  $t_{s-1}$  are concerned. Of course, the response of moths to temperature on a given evening must be affected by more preceding temperatures than that of the evening immediately antecedent. However, the correlation between temperatures of successive evenings is so high, as shown below, that a great deal

TABLE XI  
CORRELATION BETWEEN TEMPERATURES ON  
SUCCESSIVE EVENINGS

Year	Date range, inclusive, of data	Correlation coefficient
1928	July 8 - Aug. 3	+ .62
1929	July 6 - July 29	+ .70
1930	July 1 - July 20	+ .63
1931	July 4 - July 21	+ .48
1932	June 30 - July 24	+ .62
1933	June 26 - July 23	+ .59

of information on preceding conditions is conveyed by the immediately antecedent evening, and probably little more information would be added by data from further preceding evenings.

The correlation between the temperatures of successive days is shown in Table XI.

Temperature values used are those obtaining half an hour after sunset. The range of data upon which the calculations are based is indicated.

### Detailed Study of the Relation Between Activity and Evening Temperature

A detailed study of the relation between activity and evening temperature is made below. Above, the investigation by the variate-difference method showed flight and evening temperature to be consistently associated. Also, the investigation by comparing successive ratios for flight with changes in temperature showed the general nature of the relation between activity and evening temperature. In the preceding section, evidence was presented to show that one may expect the relation between activity and current temperature to vary with preceding temperature conditions.

In considering the joint effect of two or more factors upon flight of corn borer moth, it is not satisfactory to have the various effects additive. In the data under consideration, a single factor can be prohibitive of activity. Time of year can, obviously, be so prohibitive. In addition, low temperature, high wind and heavy rain may totally inhibit flight. If one should find certain functional relationships between numbers of moths and two or more factors, it would appear that the functions should be multiplied rather than added. By such an arrangement any factor can be prohibitive, and if one condition is optimal for activity, the activity varies with the other conditions. Accordingly, the form of joint solution suitable for temperature and time of year should consider the number of moths in flight the product of two functions, of date and temperature, respectively. As stated in the discussion on the nature of activity,  $\bar{n}_s = D_s \cdot A_s$ . If  $D_s = f_1(d_s)$ , where  $d_s$  is the date of the  $s^{\text{th}}$  day reckoned from an arbitrary and convenient origin, and  $A_s = f_2(t_s)$ , then  $\bar{n}_s = f_1(d_s) \cdot f_2(t_s)$ . Approximately,  $n_s = f_1(d_s) \cdot f_2(t_s)$ . The multiplicative nature of the effect of various factors appears to have been most clearly recognized by workers on the manurial value of various compounds. Thus Stewart (11) quotes the formula,

$$y = A(1 - e^{-cx})(1 - e^{-c_1 t_1}) \dots (1 - e^{-c_n x_n})$$

where  $y$  is observed yield,  $A$  is maximum yield, and each other factor to the right is the effect of some one manure. The values,  $c, c_1, \dots, c_n$  are constants to be determined and  $x, x_1, \dots, x_n$  are quantities of  $n$  different fertilizers.

The relations between activity and date must be determined separately for each year for two reasons. First, the shape and amplitude of the date relation probably varies in various years. Second, even if the shape and amplitude of the function of date were essentially the same from year to year, and hence capable of being represented by the same function, the flight occurs at different times from year to year and it is very difficult, since the date trend is obscured by the temperature relation, to decide what are the corresponding dates in different years.

The relation between activity and temperature must be determined jointly for all the years. The procedure of combining the data from the years seems to be fundamentally sound, since one would suppose the reactions of the moths to environmental conditions in any year to be a constant, specific character. Also, it would be difficult to fit a relationship with many constants from the data of a single year since in any one there were only about thirty nights when flight might occur.

For the determination of the relations between numbers of moths and, date and temperature, respectively, the methods of (7) and (6) were not satisfactory. By the method of (7) the effect of the various physical factors would necessarily be additive, whereas, in this problem, they are manifestly multiplicative. Also, there is no apparent way of combining the data from various years. In the method of Janisch the work hinges on a determination, under experimental conditions, of the temperature optimal for activity. In the field one cannot make such a determination.

A simultaneous solution for  $f_1(d_s)$  and for  $f_2(t_s)$  in the equation

$$n_s = f_1(d_s) \cdot f_2(t_s)$$

is impossible. It is impossible because one must solve for the relation between activity and date independently for each year, and for the relation between activity and temperature collectively for all years. Accordingly, solutions are made separately for the two relations. First, neglecting function of temperature, an approximation to function of date is found for each year. On the basis of this approximation to function of date, a first approximation to function of temperature, for combined years, is found. Second approximations to the functions of date and temperature are found by approximating to one relation upon the basis of the earlier approximation to the other relation.

The solution for  $f_1(d_s)$  is a polynomial,

$$a_0 + a_1 d_s + a_2 d_s^2 + a_3 d_s^3 + a_4 d_s^4$$

where  $a_0$ ,  $a_1$ ,  $a_2$ ,  $a_3$  and  $a_4$  are constants to be found. An equation of the fourth degree is considered adequate since, as was discussed in the section on choice of appropriate trend lines, one may expect flight to have only one peak in the course of a season. The solution for  $f_1(d_s)$  is made from the equation

$$n_s = (a_0 + a_1 d_s + a_2 d_s^2 + a_3 d_s^3 + a_4 d_s^4) A_s$$

where  $A_s$  is the activity, as calculated from an approximation to the relation between flight and temperature on the  $s^{\text{th}}$  day.

In the calculation of the first approximation to  $f_1(d_s)$  all values of  $A_s$  are assumed equal to one, that is, the effect of temperature is neglected. For each year an equation

$$n_s = a'_0 + a'_1 d_s + a'_2 d_s^2 + a'_3 d_s^3 + a'_4 d_s^4$$

where  $a'_0 \dots a'_4$  are the coefficients of the first approximation, is fitted

by the method of least squares. In the second approximation, for each year an equation

$$n_s = (a_0'' + a_1''d_s + a_2''d_s^2 + a_3''d_s^3 + a_4''d_s^4) A_s'$$

where  $a_0'' \dots a_4''$  are coefficients of the second approximation, and where  $A_s'$  is the approximation to the activity on the  $s^{\text{th}}$  evening as calculated from the first approximation to the relation between activity and temperature is fitted. The values for the approximations to  $f_1(d_s)$  are not shown in this paper.

The approximations to  $f_1(d_s)$  are based upon prior estimates of the activity,  $A_s$ , of each evening. For each evening one is fitting a trend value, adjusted for the appropriate activity. It is from the modified values of the trend chosen that the observations deviate to a minimum extent. Thus, if temperature is such as to inhibit flight the trend value is adjusted to a zero and the deviation of the observation is zero. Accordingly, temperature conditions, for an evening or for a period, do not affect fit of the seasonal trend. Similarly, approximations to  $f_2(t_s)$  are based upon prior estimates of the seasonal trend value,  $D_s$ , of each evening. One is, essentially, trying to find a temperature relation which will so modify the date trend that observations shall deviate from the modified values to a minimum extent.

Date trends and the relation between activity and temperature are determined from the data of the years 1928 to 1933, inclusive. The data of 1927 are omitted because they are not complete for the year, and because the methods and general volume of catch differ radically from methods and volume in other years. The data used in the calculation of approximations to  $f_1(d_s)$  cover the following dates, inclusive, in the various years: in 1928 from July 6 to August 11, in 1929 from July 3 to August 2, in 1930 from June 28 to July 26, in 1931 from June 29 to July 21, in 1932 from June 27 to July 26, in 1933 from June 23 to July 24. The curve obtained, in fitting a polynomial to date, varies according to the number of days, beyond the range of observed flight, which are included in the fitting. The ranges of dates, shown above, are chosen so that there be one evening with no flight before the first observed flight and two such evenings after the last observed flight, in each season.

The fourth degree polynomials fitted for the relation between numbers of moths in flight and date give curves with but one peak of flight, except for the data of 1931, which curve has two peaks. This effect appeared to be due to the inhibitive effect of cool temperatures about July 10 and 11, since on the second fitting when temperature effects are largely eliminated, there is only one peak.

In determining the relation between activity and temperature it would appear desirable to start with some very general curve. Relationships such as, for instance, exponentials, or curves involving steady geometrical increase of activity, appear undesirable. Accordingly, suppose that the relation may be represented by a polynomial.

A polynomial of the third degree should be adequate for the relation, since one may suppose there is only one region in the temperature range optimal

for activity. Accordingly, one may suppose activity of moths on any day varies with temperature on that day in the following manner:

$$A_s = b_0 + b_1 t_s + b_2 t_s^2 + b_3 t_s^3 \quad (1)$$

where  $b_0, b_1, b_2, b_3$ , are constants to be determined. Although, as was suggested above, the relation between activity and current temperature may be expected to be modified by temperature on the preceding evening, this modification is neglected for the moment.

Examination of Table X shows that no appreciable activity occurs at temperatures of 60° F. or lower. Knowing this, one may restrict the polynomial so that activity for  $t_s = 60^\circ$  F. is zero, and assume activity at all lower temperatures zero. If 60° F. is chosen as the origin of  $t_s$  and all fitting below 60° F. is neglected, Equation (1) becomes,

$$A_s = b_1 t_s + b_2 t_s^2 + b_3 t_s^3 \quad (2)$$

Equation (2) is constructed on the assumption that the relation of activity to temperature is constant from day to day. There is, however, as discussed above, good reason to suppose the relation varies with the temperature of the preceding evening. If the relation between temperature and activity varies continuously as the temperature of the previous evening varies, then each of the constants in Equation (2) must so vary. Accordingly, suppose,

$$b_1 = c_2 + c_3 t_{s-1}, \quad b_2 = c_4 + c_5 t_{s-1}, \quad \text{and} \quad b_3 = c_6 + c_7 t_{s-1}$$

where  $c_2 \dots c_7$  are constants to be determined. Substituting for  $b_1, b_2, b_3$ , in equation (2) one gets

$$A_s = c_2 t_s + c_3 t_s t_{s-1} + c_4 t_s^2 + c_5 t_s^2 t_{s-1} + c_6 t_s^3 + c_7 t_s^3 t_{s-1}. \quad (3)$$

Equation (3) is referred to as  $f_2(t_s)$ .

From Equation (3) one can find the relation between activity and temperature on the  $s^{\text{th}}$ , or  $(s-1)^{\text{th}}$  evenings. For  $t_s$  constant, one obtains an equation of the form

$$A_s = (c_2 t_s + c_4 t_s^2 + c_6 t_s^3) + (c_3 t_s + c_5 t_s^2 + c_7 t_s^3) t_{s-1}$$

where activity varies with  $t_{s-1}$  in the form,  $A_s = k_0 + k_1 t_{s-1}$ . The quantities  $k_0$  and  $k_1$  vary as third order equations in  $t_s$ . For  $t_{s-1}$  constant the variation of activity is a third degree polynomial in  $t_s$ .

In the calculation of the values of the coefficients in Equation (3), data from evenings when temperature was below 60° F. are not included. Such evenings are omitted, since in them activity has been assumed to be zero. Including such evenings, the data used in the calculations of approximations to  $f_2(t_s)$  cover the following periods in the various years: in 1928 from July 8 to August 1, in 1929 from July 3 to July 31, in 1930 from June 29 to July 19, in 1931 from June 30 to July 20, in 1932 from June 29 to July 20, in 1933 from June 24 to July 22.

The constants,  $c_2, c_3, \dots, c_7$ , of Equation (3) are calculated from the data by the method of least squares. The first approximation to  $f_2(t_s)$  was calculated as a second degree polynomial in  $t_s$ , that is, with four terms, and next as a third degree polynomial in  $t_s$ , that is, with six terms. The curves and the deviations from the curves from the lower order solution were very similar to those from the higher. The higher order solution was chosen for calculating the second approximation to  $f_1(d_s)$  in each year. Such a choice was not justifiable on the basis of improvement of fit; the choice was made in order to have the first and second approximations to  $f_2(t_s)$  homologous. It appeared probable that a six term solution might be more suitable than a four term for the calculation of the second approximation.

As discussed above, an approximation to  $f_1(d_s)$  is first calculated, and next, on this basis, an approximation to  $f_2(t_s)$ . The equation obtained in the first approximation to  $f_2(t_s)$  is

$$n_s = + .2092 t_s D'_s - .008,715 t_s t_{s-1} D'_s - .005,004 t_s^2 D'_s \\ + .000,307,7 t_s^2 t_{s-1} D'_s + .000,046,12 t_s^3 D'_s - .000,002,3 t_s^3 t_{s-1} D'_s$$

where  $D'_s$  is the first approximation to  $D_s$ , the mean number of moths flying under average temperature conditions, with infinite observation, over the plot. The value  $D'_s$  is obtained from the first approximation to  $f_1(d_s)$ .

The general nature of this solution for  $f'_2(t_s)$  is indicated in Fig. 2. The relation between  $t_s$ , temperature in degrees Fahrenheit in excess of 60° F.,

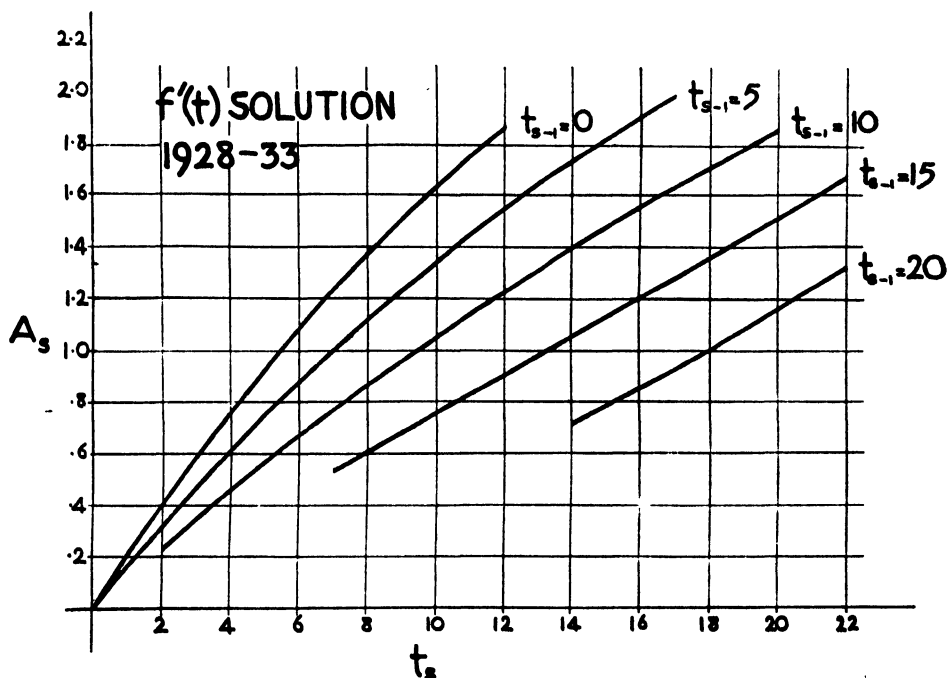


FIG. 2. Solution for the relation between temperature in excess of 60° F. and activity, first approximation. Data 1928 to 1933 inclusive.

and activity, with  $t_{s-1}$  constant, is plotted for  $t_{s-1}$  at intervals of five degrees Fahrenheit. Values of  $A'_s$  necessary for the calculation of  $f''_1(d_s)$  are found. These values are not tabled. They are, however, similar to those tabled for  $f''_2(t_s)$ , which are shown below.

The activity found under average temperature conditions receives in the calculation of  $f_2(t_s)$  the value of 1.00. Of course, the average is weighted by the number of moths to be expected from the date trend. All other values of activity are referable to this value.

Upon the basis of the second approximation to  $f_1(d_s)$ , a six-term, second approximation to  $f_2(t_s)$  is calculated. The equation obtained is

$$n_s = +.160,861 t_s D''_s + .003,201,29 t_s t_{s-1} D''_s + .005,905,40 t_s^2 D''_s \\ - .001,567,61 t_s^2 t_{s-1} D''_s - .000,504,613 t_s^3 D''_s + .000,068,168,7 t_s^3 t_{s-1} D''_s$$

where  $D''_s$ , the second approximation to  $D_s$ , is obtained from the second approximation to  $f_1(d_s)$ .

When Equation (3) is put in the form

$$n_s = (c_2 + c_3 t_{s-1}) t_s + (c_4 + c_5 t_{s-1}) t_s^2 + (c_6 + c_7 t_{s-1}) t_s^3$$

and  $t_{s-1}$  is chosen any constant value, an equation for  $n_s$  in terms of  $t_s$ , of the type

$$n_s = b_1 t_s + b_2 t_s^2 + b_3 t_s^3$$

is obtained. Such coefficients,  $b_1$ ,  $b_2$ , and  $b_3$ , are calculated for all values of  $t_{s-1}$  from 57° F. to 82° F., inclusive. From the twenty-six resulting equations values of the activity for various values of  $t_s$  and  $t_{s-1}$  are calculated. These values are shown in Table XII.

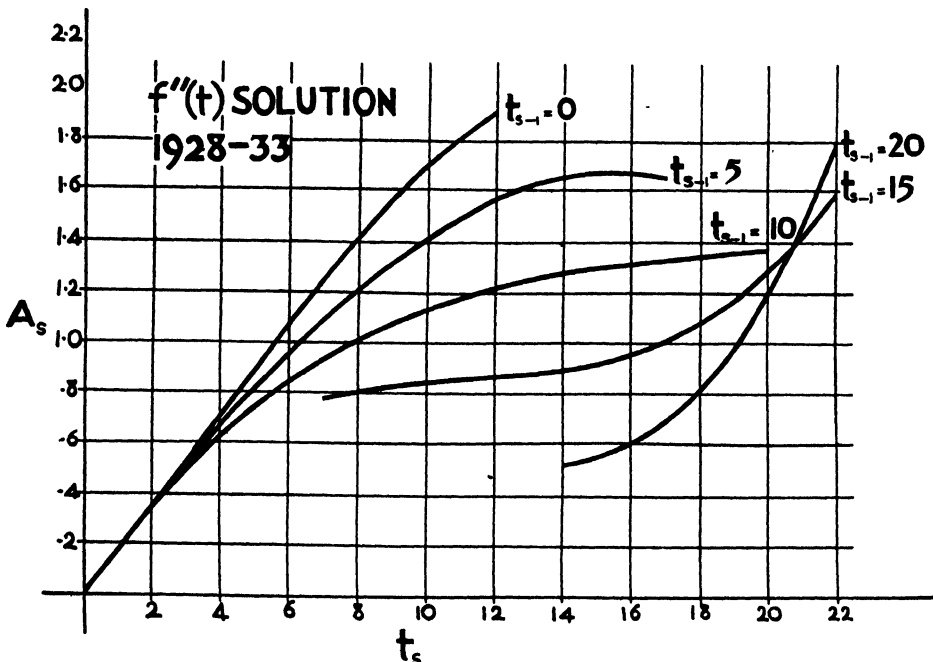


FIG. 3. Solution for the relation between temperature in excess of 60° F. and activity, second approximation. Data 1928 to 1933 inclusive.

TABLE XII  
SECOND APPROXIMATION TO  $f_2(t_s)$

$t_s$	$t_{s-1}$																78	79	80	81	82
	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77
60	0.00																				
61	.16	.16	.16	.17	.17	.17	.17	.17	.17	.18	.18	.18	.18								
62	.34	.34	.34	.34	.34	.34	.34	.34	.35	.35	.35	.35	.35	.35							
63	.53	.53	.52	.52	.52	.52	.51	.51	.51	.51	.50	.50	.50	.49							
64	.73	.72	.71	.71	.70	.69	.68	.67	.67	.66	.65	.64	.63	.63	.62	.61					
65	.93	.92	.90	.89	.87	.86	.84	.83	.82	.80	.79	.77	.76	.74	.73	.71	.70				
66	1.14	1.11	1.09	1.07	1.05	1.02	1.00	.98	.96	.93	.91	.89	.87	.84	.82	.80	.78	.75			
67	1.34	1.30	1.27	1.24	1.21	1.18	1.15	1.12	1.09	1.06	1.03	.99	.96	.93	.90	.87	.84	.81	.78	.77	
68	1.49	1.45	1.41	1.37	1.33	1.29	1.25	1.21	1.17	1.13	1.09	1.05	1.01	.97	.93	.89	.85	.81	.78		
69	1.66	1.61	1.56	1.51	1.46	1.41	1.36	1.32	1.27	1.22	1.17	1.12	1.07	1.03	.98	.93	.88	.83	.78	.77	
70		1.75		1.69	1.64	1.58	1.52	1.47	1.41	1.36	1.30	1.24	1.19	1.13	1.07	1.02	.96	.90	.85	.79	.73
71				1.81	1.75	1.68	1.62	1.56	1.49	1.43	1.37	1.30	1.24	1.18	1.11	1.05	.98	.92	.86	.79	.73
72				1.91	1.84	1.77	1.70	1.63	1.56	1.49	1.42	1.35	1.28	1.21	1.14	1.07	1.00	.94	.87	.80	.73
73					1.91	1.83	1.76	1.69	1.61	1.54	1.47	1.39	1.32	1.25	1.17	1.10	1.02	.95	.88	.80	.73
74						1.87	1.80	1.72	1.65	1.57	1.50	1.42	1.35	1.27	1.20	1.12	1.04	.97	.89	.82	.74
75							1.81	1.74	1.67	1.59	1.52	1.44	1.37	1.29	1.22	1.14	1.07	.99	.92	.84	.77
76							1.74	1.66	1.59	1.52	1.45	1.38	1.31	1.24	1.17	1.10	1.03	.96	.88	.81	.74
77								1.64	1.58	1.52	1.45	1.39	1.33	1.26	1.20	1.13	1.07	1.01	.94	.88	.82
78									1.55	1.50	1.44	1.39	1.34	1.29	1.23	1.18	1.13	1.08	1.02	.97	.92
79										1.43	1.39	1.35	1.31	1.28	1.24	1.20	1.16	1.13	1.09	1.05	.99
80											1.37	1.35	1.33	1.31	1.30	1.28	1.26	1.24	1.22	1.21	1.19
81												1.40	1.40	1.40	1.40	1.40	1.40	1.40	1.40	1.40	1.40
82													1.40	1.40	1.40	1.40	1.40	1.40	1.40	1.40	1.40

All values of  $f_2(t_s)$  for  $t_s < 60^\circ F.$  are 0.00.

From Table XII the relation between  $t_s$  and activity is plotted in Fig. 3, for  $t_{s-1}$  constant at intervals of five degrees Fahrenheit. Figs. 2 and 3 are comparable. The second approximation to  $f_2(t_s)$  gives very similar curves to the first approximation.

In order to evaluate a calculated relation, deviations, squared, of observations about the relation are summed. This sum is compared with the summed deviations squared about the mean of the observations, in each year, over the range of dates used in calculating the relation. The reductions in sums effected by the two approximations to the relation  $f_1(d_s)$  are first compared. Secondly, the reductions effected by the approximations to  $f_2(t_s)$  are compared.

The quantities given in Table XIII, as the sums of squares about the approximations to the date relations in each year, are readily calculated from the summations made in fitting the relations. The sum of squares about the first approximation to the relations is

$$\sum_s n_s^2 - a_0' \sum_s n_s - a_1' \sum_s n_s d_s - a_2' \sum_s n_s d_s^2 - a_3' \sum_s n_s d_s^3 - a_4' \sum_s n_s d_s^4.$$

The sum of squares about the second approximation to the relation is

$$\sum_s n_s^2 - a_0'' \sum_s n_s A_s' - a_1'' \sum_s n_s d_s A_s' - a_2'' \sum_s n_s d_s^2 A_s' - a_3'' \sum_s n_s d_s^3 A_s' - a_4'' \sum_s n_s d_s^4 A_s'.$$

TABLE XIII

SUMMED DEVIATIONS SQUARED ABOUT APPROXIMATIONS TO  $f_1(d_s)$  AND VARIANCE FOR EACH YEAR

Year	Days in range of dates	Sum of squares about mean	Sum of squares about $f_1(d_s)$	Sum of squares about $f_1''(d_s)$
1928	37	1702.0	889.6	685.5
1929	31	465.7	307.2	214.2
1930	29	3750.1	2803.3	2320.6
1931	23	224.6	143.5	117.8
1932	30	1319.0	590.2	339.0
1933	32	3774.2	1783.4	679.7

It is apparent that both approximations are profitable, since in all years the sum of deviations, squared, about the first approximation to the function is much smaller than the variance. The corresponding sums for the second approximation are all a little smaller than those for the first approximation. It should, however, be noted that the second approximation to the function of date is made upon, and including, the first approximation to the function of temperature.

In the same way as the reduction in sums of deviations squared was calculated about the functions of date, so are sums of deviations squared calculated about the functions of temperature. The results are as shown in Table XIV. The range of dates upon which temperature calculations are based is slightly shorter than that used in the calculations on date. The sum

of squares about the first approximation to the relation is:

$$\sum_s n_s^2 - c_2' \sum_s n_s t_s D_s' - c_3' \sum_s n_s t_s t_{s-1} D_s' - c_4' \sum_s n_s t_s^2 D_s' - c_5' \sum_s n_s t_s^2 t_{s-1} D_s' \\ - c_6' \sum_s n_s t_s^3 D_s' - c_7' \sum_s n_s t_s^3 t_{s-1} D_s'.$$

The sum of squares about the second approximation to the relation is

$$\sum_s n_s^2 - c_2'' \sum_s n_s t_s D_s'' - c_3'' \sum_s n_s t_s t_{s-1} D_s'' - c_4'' \sum_s n_s t_s^2 D_s'' - c_5'' \sum_s n_s t_s^2 t_{s-1} D_s'' \\ - c_6'' \sum_s n_s t_s^3 D_s'' - c_7'' \sum_s n_s t_s^3 t_{s-1} D_s''.$$

TABLE XIV

SUMMED DEVIATION SQUARED ABOUT APPROXIMATION TO  $f_2(t_s)$  AND VARIANCE, FOR EACH YEAR

Year	Days in range of dates	Sum of squares about mean	Sum of squares about $f_2(t_s)$	Sum of squares about $f_2''(t_s)$
1928	25	1378.0	728.3	654.0
1929	29	439.8	313.9	215.4
1930	21	3405.0	2612.8	2177.9
1931	21	203.1	95.4	121.7
1932	22	1028.6	299.5	269.5
1933	29	3432.8	717.9	380.5

It is apparent that the first approximation to  $f_2(t_s)$  greatly reduces the sum of deviations squared. The second approximation effects some reduction over the first in data of all years, except of 1931.

From these data on corn borer moths it is apparent that activity increases, in general, with current temperature. The activity associated with any current temperature is affected by preceding temperatures. The rate at which activity increases with increase in current temperature depends upon preceding temperature. There are indications that the rate at which activity increases with rise in temperature tends to decrease when temperature becomes very high relative to that of the preceding night. The temperature at which activity is a maximum depends upon previous temperature conditions. The existence and general nature of the temperature effects conform with findings (14) on the effect exerted by change in temperature upon rate of development.

The labor involved in making the fits by least squares, for two approximations to  $f_1(d_s)$  and  $f_2(t_s)$ , was great. The method of solution used was that of Gauss (1). Use of this method reduced the work a great deal.

It is desirable to see how closely the values estimated from  $f_1''(d_s)$  and  $f_2''(t_s)$  agree with the observed magnitude of flight. Let  $\tilde{n}_s$  be, as previously, the estimate of flight over the laboratory plot on the  $s^{\text{th}}$  evening, and  $\tilde{n}_s = f_1''(d_s) \cdot f_2''(t_s)$ . In Fig. 4, magnitude of the values of  $\tilde{n}_s$  are shown as narrow, solid columns, and of  $n_s$  as broad hollow columns. In calculating values of  $\tilde{n}_s$  a few cases occurred where the values,  $t_s$ ,  $t_{s-1}$ , were one or two degrees outside the range of Table XII. In these cases values,  $f_2''(t_s)$ , were obtained by extrapolation. However, for July 31, 1929, when  $t_{s-1}$  was 66° F. and

$t_s$  was 80° F., no value for  $f_2''(t_s)$  could be found. Except for the deletion of this abnormal case, the limits of the range of dates, considered for each year in Fig. 4, are those used in finding  $f_2''(t_s)$ . In the figure the limits of the period are indicated by thin vertical lines.

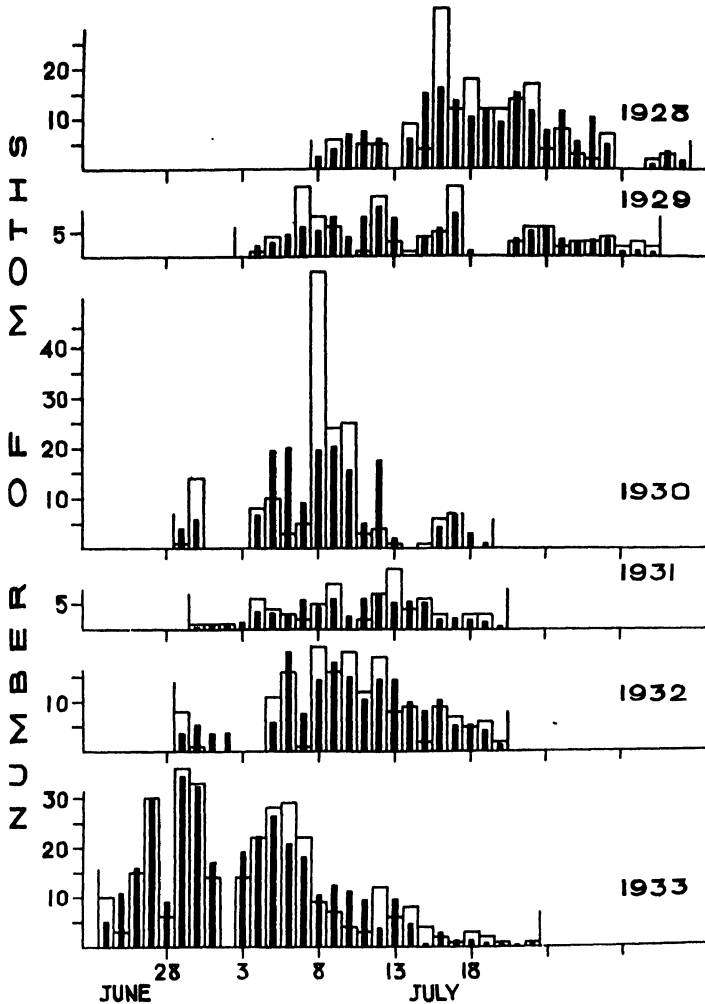


FIG. 4. Observed flight, in hollow columns, and values estimated from date and temperature relation, in solid columns.

From Fig. 4, it can be seen that there is general agreement between the magnitude of  $\bar{n}_s$  and  $n_s$ . There is, however, also considerable variation between the estimated and observed values. The situation is examined more closely in Table XV. In this table the estimated values are grouped for ranges of one moth and against each range observed values are entered. For ranges with more than one entry, mean,  $\bar{x}$ , and variance,  $s^2$ , of the observations are calculated.



From Table XV, it can be seen that in each range the mean of the observed values varies closely with the magnitude of the estimated value. Also, the variance in each range varies, as was anticipated in the preceding section on the chance error of observations, with the magnitude of the estimated value. In that section it was shown that the variance, if ascribable only to random variability, would be approximately equal to the estimated value. In fact, there are two sources of variability, random variability and the effect of conditions other than date and temperature, within each range. Accordingly, the variance is larger than estimated values in Table XV. The proportional difference between estimated value and variance is greatest when the estimated value is great, for then temperature conditions tend to be optimal and, from the multiplicative nature of the joint effect of various factors, other physical conditions must effect flight greatly.

### Conclusions

It is found that, on a given evening, variation in numbers of moths over an area is distributed with the standard deviation of these chance variations equal to the square root, approximately, of the observation. This relationship should be of use in handling data from such sources as surveys or traps. Thus, one should be able, neglecting seasonal trend, to judge whether observations made on successive evenings differ significantly. Since this preliminary study shows flight of corn borer moths to fluctuate in a nearly parallel manner over various areas, it is reasonable to seek causes, common to a district, of fluctuations.

From the relation between flight of the corn borer moth and temperature, the importance of weather during a season may be estimated and the probable success in extension of geographical distribution forecast. The relationship shows that there is, within the temperature range where flight is possible, so much adaptability in response, on the part of the moths, that it is improbable, provided temperature is not consistently very low, that paucity of moths is ascribable to the effect of temperature on flight. The relationship, also, foreshadows the success of the borer as it spreads, in North America, west and south. On the one hand, there is no ground for hope that the high night temperatures of the southern Mid-west will prove detrimental to flight of the moth. On the other hand, cool evenings in the high altitudes of the West and in the neighborhood of the Pacific Ocean, will act against the species.

An examination was made of the correspondence, each night, between magnitude of flight, as observed, and as estimated from the determined relations of flight to date and to temperature. For narrow ranges of estimated values the mean of the observed values varied closely with the magnitude of the estimated value. Also, the variance in each range varied with the magnitude of the estimated value. The effect of conditions other than date and temperature was greatest when the estimated value was great, for then temperature conditions tended to be optimal and, from the multiplicative nature of the joint effect of various factors, physical conditions other

than temperature affected flight greatly. This examination indicates that an estimation of flight over a small area is necessarily inexact on account of chance variation. However, for a wider area the favorableness of temperature conditions may be estimated.

The methods used in studying flight of the corn borer moth may be applied in studying activities of other species of insects. Such application would determine the importance of various physical conditions. Under what conditions a given activity may be expected to be great, or in what new regions the insects may succeed, could be judged.

### Acknowledgments

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## STUDIES ON THE BIONOMICS AND CONTROL OF THE BURSATE NEMATODES OF HORSES AND SHEEP

### V. COMPARISONS OF THE LETHAL EFFECTS OF SOME NON-NITROGENOUS FERTILIZERS ON THE FREE-LIVING STAGES OF *SCLEROSTOMES*<sup>1</sup>

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#### Abstract

The effect on the free-living stages of *sclerostomes* of some non-nitrogenous artificial fertilizers, containing potash, phosphoric acid or calcium, is discussed. Of these fertilizers kainit has most practical advantages. Under the conditions of the experiments, which are otherwise ideal for the survival of the larvae, one part of kainit to 23 parts of fresh horse feces is necessary to sterilize them. The proportions in which the other fertilizers must be mixed are:—Muriate of potash 1: 17, (potassium chloride, one of the main constituents of the previous fertilizer, is rather more lethal); carbonate of potash 1: 13; sulphate of potash 1: 5. Superphosphate (20%) sterilized when mixed at 1: 5, and 16% superphosphate required 2: 5. Basic slag and raw rock phosphate (Florida) had no sterilizing value. Lime, in spite of its reputation as a sterilizing agent for many pests has, when mixed with fresh feces, little effect on the free-living stages of *sclerostomes*.

When urine is not available to sterilize manure containing the free-living stages of *sclerostomes*, it may be preferable, for some crops and on some soils, to treat the manure with a non-nitrogenous fertilizer rather than with a nitrogenous one. This paper discusses the values of kainit, muriate of potash, potassium chloride, carbonate of potash and sulphate of potash, of 20% and 16% superphosphate, basic slag, and Florida raw rock phosphate, of quick lime, hydrated lime and ground limestone.

Many fertilizers, whether mined as salts, made synthetically or obtained as a by-product from the manufacture of other materials, may contain impurities, some of which may be highly lethal, e.g., iodine salts, which are lethal even in 0.1% solutions. When these impurities have a high lethal value but are not constantly found in any fertilizer, slight variations must be expected in the results reported with that fertilizer. For this reason, potassium chloride, which is the main constituent of kainit and muriate of potash, has been tested in comparison with those fertilizers because they contain many other salts in smaller quantities.

Potassium xanthogenate, potassium permanganate, potassium iodide, potassium iodate, potassium hydroxide, trisodium phosphate, calcium hypochlorite and calcium borate will be discussed in subsequent papers.

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Potassium nitrate, diammonium phosphate and some nitrogenous fertilizers containing calcium have been discussed in a previous paper (22).

The technique used to obtain the data for this paper was that described in the second paper of this series (21) except that the larvae from all cultures made after July 31, 1936 (Control Numbers CXLIII *et seq.*) have only been drawn from the funnels once, always on the tenth day. The previous five thousand cultures have shown that only in very rare cases has the number of larvae obtained after ten days' extraction been greater than the expected error of counting, etc. During the ten-day extraction process, the water in the top of the funnels has always been changed three or four times, warm water being used for replacing that poured off. During further tests to determine whether this technique was likely to result in many larvae being accidentally thrown away, it was found that the average rate of fall of larvae in water is about one foot per hour, although very active larvae may remain swimming without losing height for many hours.

A minor modification has also been introduced to facilitate counting. When the larvae are numerous, 10 cc. of diluted fluid has regularly been measured into the counting dish instead of 20 cc. Further, instead of the usual dilution from 40 cc. to 80 cc., dilutions from 40 cc. to 100 cc. have been made on the basis of a rough estimate of the number of larvae, made by viewing the fluid against a bright light.

J. Leiper (12, (and personal communications)) suggests that the description of the technique used to obtain the data for these papers needs elaborating, especially to make it clear whether the chemicals have acted on the eggs or on the subsequent larval stages. Some additional notes on technique are accordingly included in this paper. In the two previous papers, this and the subsequent papers, data relate to the effect of fertilizers or chemicals on "fresh" feces, *i.e.*, feces in which the eggs have only had sufficient time to make a very little development before being treated; the feces are treated within about four hours of their being passed, so that all the chemicals have been able to act on the eggs. Any chemical which was not rapidly altered by contact with the feces or by bacteria has acted also on the free-feeding larvae. The more slowly changing or slowly evaporating chemicals, those which do not change or evaporate, and those of which more was added than could be changed by the limited amount of feces present, have acted on the infective larvae also.

It is, of course, obvious, but should perhaps be noted in connection with this technique, that when a chemical allows the larvae to reach the infective stage and then causes their death, only those on the outside of the culture or on the walls of the glass container can be washed off and counted.

The determination of whether or not extended larvae were dead has been made by one or more of the following methods. Larvae, especially those which were extended or slightly curved, were considered dead, if they appeared, under the binocular microscope ( $\times 20$ ), to have lost all their internal structure. "Staining" of the larvae by the chemical was found to be no certain indication of death. When death was not obvious, the larvae

were touched with a needle, preferably at a point situated about one-third of their length from the head. If dead, they frequently were easily cut or broken, while if alive they usually moved. By using a combination of these two methods it has generally been comparatively easy to decide whether many of the larvae were dead, but very occasionally it has been necessary to leave the larvae on the lighted microscope stage for some minutes or even for a few hours, in order to ascertain whether they were capable of movement.

Some chemicals cause larvae to exsheath without necessarily causing their death. Since these larvae are believed to be less likely to survive either weather conditions when the manure is spread, or putrefaction, etc., when the manure remains in a heap, they have not been counted; when numerous their presence has been noted by an E.

In describing the strength of the various solutions used, the quantity of chemical has always been noted first and the quantity of water last; solid chemicals have been measured by weight, fluids by volume.

When gases are being tested, or when the chemical or feces liberate gases, the volume of the container may be as important a factor as the quantity of feces used. All the cultures reported in these papers were made in glass containers of about 550-cc. capacity. During the making of the cultures reported in this and previous papers, rubber rings have not been used on the lids of the glass containers. In some tests of gases, which will be described in subsequent papers, the absolute necessity of not allowing the jars containing cultures to be airtight was shown. Some control cultures put into jars with a rubber ring under the lid have yielded very few larvae or none, even when the cultures were kept airtight for only one or more weeks and sufficient air for development has been admitted some days before extraction of any larvae.

It was previously noted (21) that the feces have always been collected from one stable where the treatment of all the horses is very similar. However, the large differences in the number of larvae found in the different controls may be partially accounted for by the variations in the ages of the horses from which the feces have been collected, as Foster (9) has shown that horses over 15 years old become less liable to sclerostomiasis. The ages of the horses from which these feces have been collected have varied from about six to over 20 years. The great majority of the cultures have been made from feces collected at mid-day, at which time egg yields are stated by Cornils (3) to be most uniform.

Lime, phosphatic and potash fertilizers have been tested as controlling agents for a number of animal pests in manure and soil. Generally the results have not been satisfactory. For instance, it was found that kainit, muriate of potash and acid phosphate, even combined with nitrogenous fertilizers, were without promise as controlling agents for fly larvae in manure (2). The results obtained when kainit was used both as a repellent and as a lethal agent against wire worms in soil were contradictory (10). Morris (15) has shown that manuring with artificial fertilizers has little effect on

the invertebrate population of the soil, while dung causes a considerable increase in numbers. Numerous workers, especially during the last few years, have tested various non-nitrogenous fertilizers against plant nematodes. The majority of these results have been difficult to interpret, because the extra essential plant food or the correction of the phosphoric-acid-potash balance has strengthened the plants or increased crop yield without necessarily reducing the nematode population. To a corrected phosphoric-acid-potash balance, Blenkinsop (1) ascribed the main value of sulphate of potash, which on other soils was found by Edwards (6, 7) not to decrease the degree of infection of potatoes by *Anguillulina dipsaci*, when 672 lb. or even 1,232 lb. per acre was used. Under the same conditions 672 lb. muriate of potash and 3,360 lb. ground quicklime per acre were also found to have no control value. The same author (5) has also shown that quicklime has hardly any value against *Heterodera schachtii*. Again, the manurial value to the plant rather than control of the nematodes is suggested by the results of Walton, Ogilvie and Brian (26), which included quicklime followed by urea. Ogilvie and Mulligan (19) confirm the report that sulphate of potash was ineffective against *Heterodera*. Hurst and Triffitt (11) have also shown that superphosphate, basic slag, muriate of potash, kainit and sulphate of potash have no lethal value against *Heterodera schachtii*, but that potassium sulphate, in large quantities, may make the plant resistant to its attack. Morgan (14) also obtained results which suggested that potash, but not lime, might help against potato eelworms. The evidence of Putnam and Chapman (23) shows that up to 1,500 lb. per acre of superphosphate did not control *H. schachtii* in Ontario. In fact the damage caused by the nematode could outweigh the fertilizing value of the superphosphate.

Free of soil, it has been shown that *Anguillulina dipsaci* is not killed by a two-hour immersion in a 2% solution of potassium sulphate (17).

However, the results obtained with some of these fertilizers against the free-living stages of nematodes of animals are more nearly comparable to the tests described here. The results with lime are somewhat contradictory. Lime has been tested with promising results for the control of hookworms. In China (4), it was mixed with night soil in large and small scale cultures; while in Japan (13) it has been tested on ground contaminated with hookworms. However, 224 lb. of lime per 120 sq. yd. was not effective against the non-bursate nematodes of poultry in Scotland (16).

Against sclerostome eggs and larvae, some non-nitrogenous fertilizers, including lime, kainit, potash salts, superphosphate (acid phosphate) and basic slag (Thomas meal) have been tested in Germany, both in the presence of feces and in their absence. Nöller and Schmid (18) obtained promising results with a 1% caustic lime solution and with the same fertilizer dry. Basic slag, dry, and as a 1% solution, was also rapidly effective. A 1% solution of superphosphate affected the larvae soon, but less rapidly. Kainit, dry, and as a 1% solution, immobilized the larvae in a few days, but potash salts were less effective. Enigk (8) has reported on the effects of a large

number of fertilizers on both eggs and larvae. He states that in dilute milk of lime, eggs developed embryos, and that in 2% solutions of basic slag, superphosphate, and kainit, they became infective larvae. When the eggs were in feces, he found that a 1:10 solution of caustic lime was effective but that a 1:20 solution was not, nor was a 2% solution of kainit or basic slag. He also found that a 1% solution of kainit was lethal to infective larvae in 29 days and a 2% solution in 17 days, that a 1% basic slag solution was lethal in 14 days and a 2% solution in 10 days, while the same strengths of superphosphate solution took 16 and 8 days; 1:20 solution of slaked lime killed the majority of the larvae in two hours. The effect of some chemicals on infective larvae placed on grass was also investigated and Enigk found that in three days thin milk of lime, 1:20, killed 60% to 75%; that a 1% solution of superphosphate killed 20%–30%; while 1% basic slag and 1% kainit solutions killed only 10%–20%. Richters and Frischbier (24) reported that milk of lime was effective, but stressed the necessity of intimate mixing with the feces.

Preliminary tests with most of the fertilizers discussed in this paper have been made with feces containing eggs or larvae on grass (20). These preliminary tests suggest that carbonate of potash, sulphate of potash, muriate of potash, kainit, and 20% superphosphate were not really effective against infective larvae when used at the rate of 3,000 lb. per acre. Hydrated lime was one of the most effective fertilizers used against the infective larvae, although when applied to the earlier stages it produced less favorable results.

### *Kainit*

### POTASSIC FERTILIZERS

Fig. 1 shows the effects of kainit on the numbers and condition of ensheathed infective sclerostome larvae obtained from 40 gm. of horse feces treated with the fertilizer within four hours of being passed. Kainit is the least rich in  $K_2O$  of the potassic fertilizers discussed in this paper (25), although the sample tested was of a higher grade than usual—20%. The chief sources of supply are the mines of Alsace-Lorraine and western Germany. The fact that kainit is the least concentrated potassic fertilizer means it is also the least expensive per ton; as it is the most lethal, weight for weight, it is, therefore, considerably the cheapest non-nitrogenous fertilizer to use as a lethal agent. In addition, when mixed with manure it fixes the nitrogen and so prevents its loss as ammonia.

Kainit was tested dry in quantities ranging from  $\frac{1}{2}$ % to 20% of the feces by weight. Sterilization against sclerostomes was effective in any culture which was treated with 2.0 gm. or more, *i.e.*, more than 5% of dry kainit is lethal. However, in some cultures 1.0 and 1.5 gm. were effective.

With a 1:2 aqueous "solution" 4.0 cc. produced sterilization; this quantity contains slightly over 4% of the weight of feces. In some cultures treated with larger amounts, some larvae reached the infective stage, but their subsequent death rate was high.

The results obtained with a 1:4 solution indicate that 7.5 cc., or 4.4%, is necessary to effect sterilization.

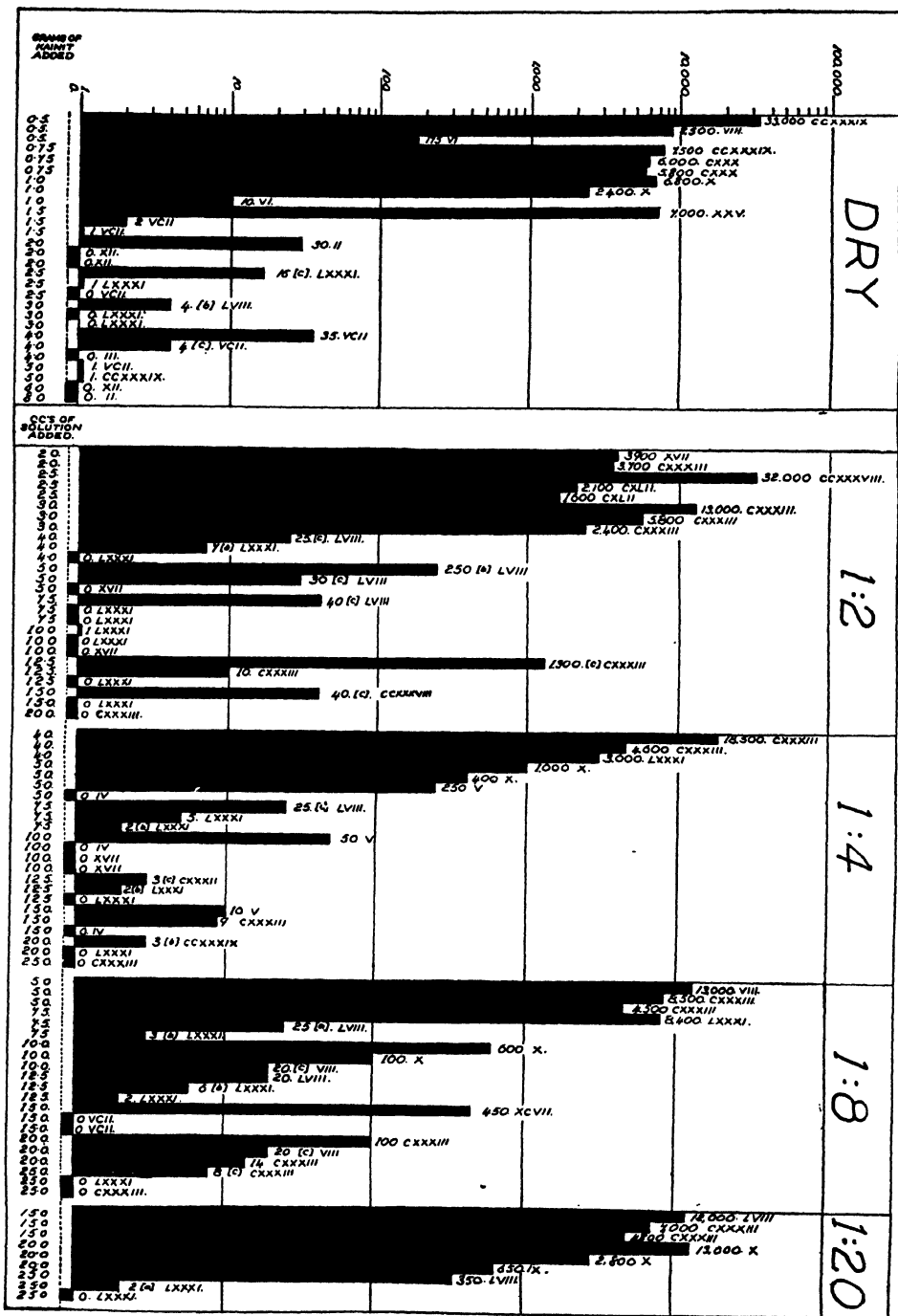


FIG. 1. Results of tests with kainit, dry and in solution, on 40-gm. cultures of fresh horse feces.

Roman numerals refer to the controls shown in Table 1.

a. after a number means that all, or practically all, the larvae were dead;

b. that a considerable proportion of the larvae were dead;

c. that a few of the larvae were dead;

d. that the culture included some sclerostome larval sheaths which were not counted.

The letters have the same significance in subsequent figures.

With a 1:8 solution the results were more irregular. Two cultures were practically sterilized by 7.5 cc., but 600 and 100 larvae were isolated from two others treated with 10.0 cc., and 450 and 100 larvae from cultures treated with 15.0 cc. and 20.0 cc. Averaging these results it can be expected that 12.5 cc., which is equivalent to slightly over 3.75%, is the lethal quantity of fertilizer applied as a 1:8 solution.

With a 1:20 solution, 25.0 cc., the largest quantity tested, almost produces sterilization. In two cultures it did, although 350 larvae were isolated from a third. This volume contains about 2.1%.

These figures suggest that about 4.75% by weight of kainit to fresh feces is effective as a sterilizing agent against sclerostomes.

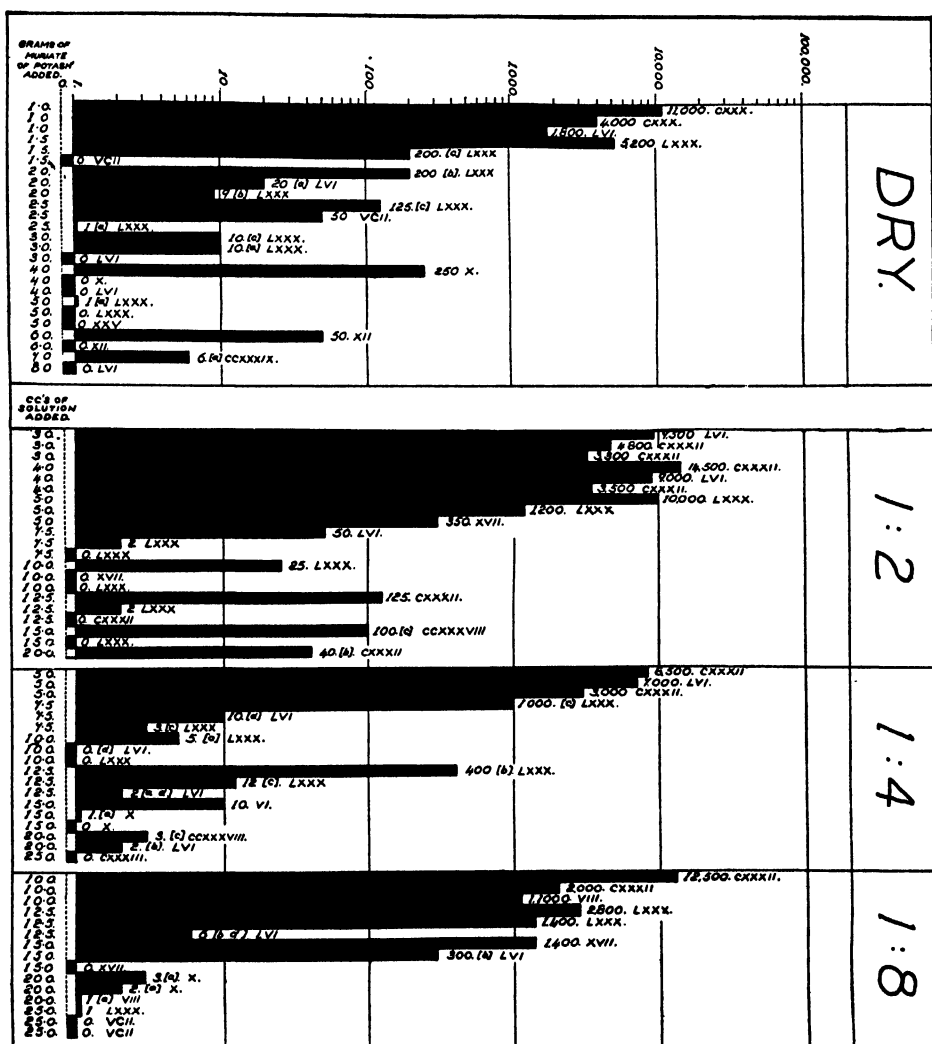


FIG. 2. Results obtained with muriate of potash, dry and in solution.

Magnesium sulphate, which with potassium chloride, is one of the main constituents of kainit, will be discussed in a future paper; its lethal value is low.

### Muriate of Potash

Fig. 2 shows the results obtained with muriate of potash. This fertilizer is mined in the same localities as kainit, but is considerably richer in potash, containing about 50%  $K_2O$  (25).

Dry muriate of potash was tested in quantities ranging from 1.25% to 20% of the feces, by weight. One culture was completely, and another

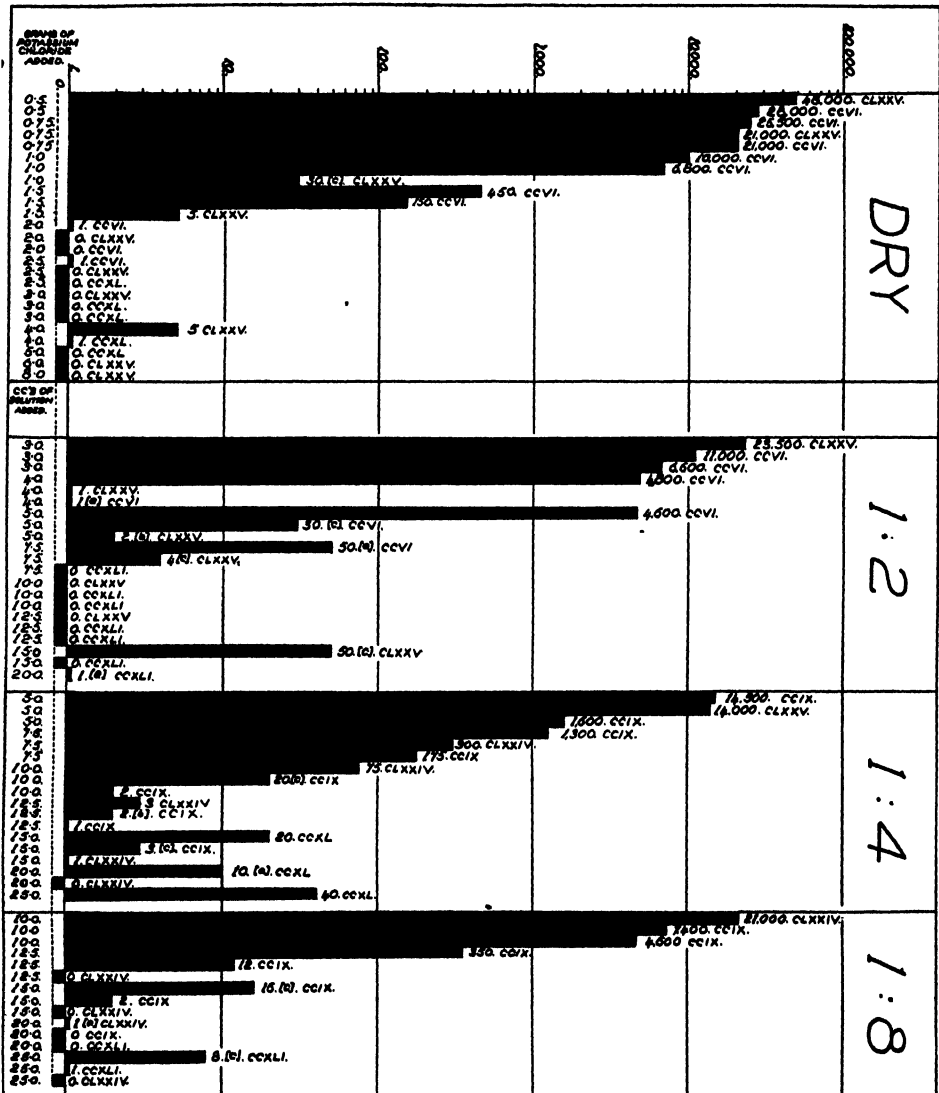


FIG. 3. Results obtained with potassium chloride, dry and in solution.

partially, sterilized by 1.5 gm. The majority of cultures were completely or almost sterilized by 2.0 gm. or more, although 250 and 50 larvae escaped in cultures treated with 4.0 and 6.0 gm. These results suggest that it may be expected that 2.0 gm. or 5% will cause sterilization.

The results for all solutions are extremely difficult to interpret owing to their irregularity. Although 50, 25, 125, 100 c. and 40 b. larvae were recovered from cultures treated with 7.5 cc., 10.0 cc., 12.5 cc., 15.0 cc., and 20.0 cc. of a 1:2 solution, the fact that other cultures made with 7.5 cc. and more of a 1:2 solution were sterilized suggests that about 7½% is the lethal quantity.

Results from cultures treated with a 1:4 solution confirm those from the 1:2 solution. Both 7.5 cc. and 12.5 cc. sterilized two out of three cultures, while 10.0 cc. sterilized all three cultures; the last quantity contains 5.7% of the weight of feces.

One culture was sterilized by 12.5 cc. of a 1:8 solution, and one completely and one almost sterilized by 15.0 cc.; the cultures treated with 20.0 cc. and 25.0 cc. were also completely sterilized; 20.0 cc. of a 1:8 solution of muriate of potash contains 6% of the weight of the feces treated.

The larger quantities of a 1:20 solution caused reduction in the numbers of the larvae but not sterilization.

These results suggest that about 6% of muriate of potash is required to sterilize fresh feces.

### *Potassium Chloride*

Fig. 3 illustrates the results obtained with pure potassium chloride.

It was tested dry in quantities of 0.83% and more. Two grams, or 5%, is necessary to ensure sterilization, although one culture was practically sterilized by 1.0 gm. and another by 1.5 gm.

When applied as a 1:2 solution this chemical completely or almost sterilized two out of three cultures treated with both 4.0 cc. and 5.0 cc., but in the other cultures, 4,700 and 4,600 larvae survived. Fifty larvae also reached the infective stage in one culture treated with 7.5 cc., but subsequently died; 5.0 cc. is equivalent to 5%.

The numbers of larvae isolated from the cultures treated with a 1:4 solution indicate that 7.5 cc. considerably reduces the number of larvae which survive, that 10.0 cc. almost causes sterilization and that 12.5 cc. does so; the latter quantity contains about 7.0%.

A 1:8 solution was comparatively more effective, as 12.5 cc. completely sterilized one culture, almost sterilized another, and only allowed 350 larvae to survive from the third culture; 15.0 cc., equivalent to 4.5%, was effective.

No cultures were sterilized by 10.0 cc. to 25.0 cc. of a 1:20 solution, but a considerably greater reduction in the number of the larvae occurred in the cultures treated with 25.0 cc. than could be accounted for by the 50% which has to be allowed for decrease in numbers caused by the addition of excessive moisture (21). This quantity of solution contains less than 3.1% of potassium chloride.

These small-scale cultures suggest that slightly more than 5% of potassium chloride must be added to fresh horse feces to kill the free-living sclerostomes.

### Carbonate of Potash

Most of the carbonate of potash used as fertilizer is obtained as a by-product of the sugar beet industry (25). The sample used for the cultures illustrated in Fig. 4, however, was somewhat purer than that usually supplied as a fertilizer, which averages between 40% and 50% potash as  $K_2O$ .

Tested dry, one culture was sterilized by 2.0 gm., but the smallest number of larvae isolated from any of the three cultures treated with 2.5 gm. was 1,700. From the cultures treated with 3.0 gm. the greatest number of

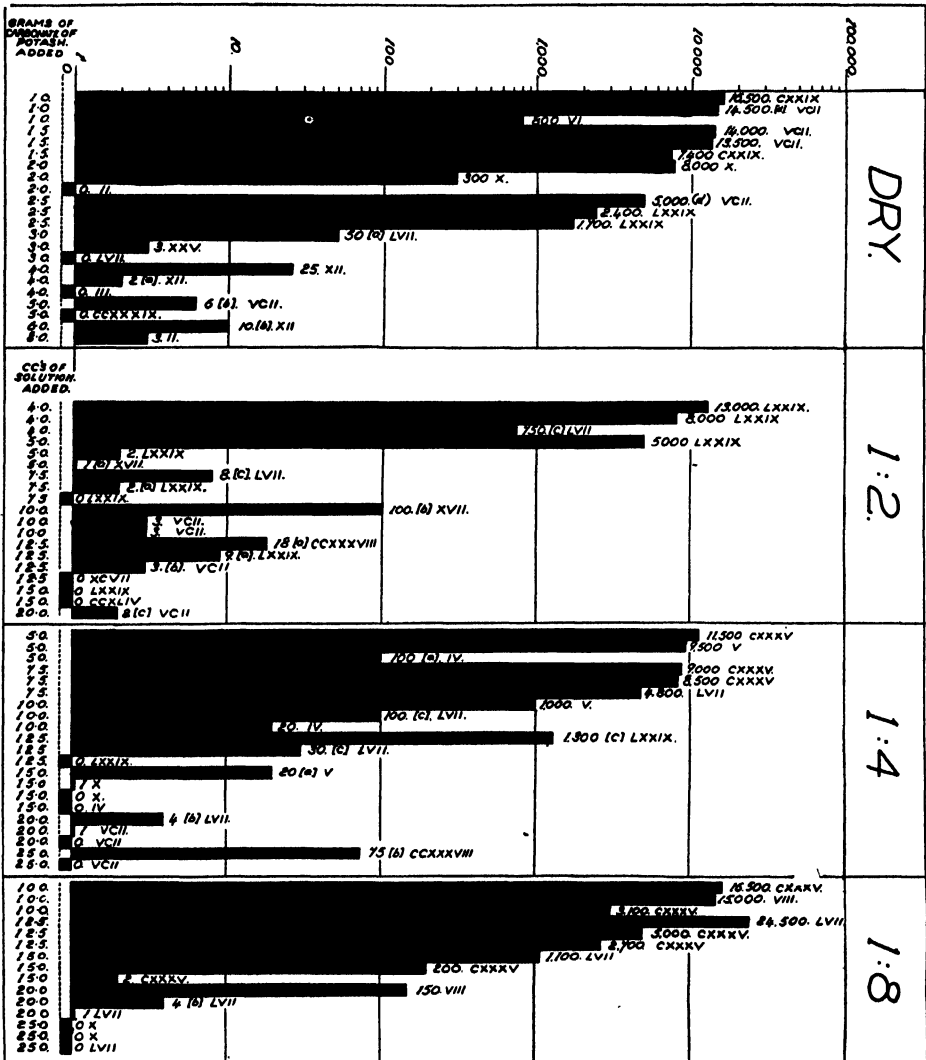


FIG. 4. Results obtained with carbonate of potash, dry and in solution.

larvae recovered was 50—all dead. Three grams is equivalent to 7.5% of the weight of the fresh feces treated.

The results obtained with solutions were irregular. One culture was sterilized by 2.0 cc. of 1:2 solution but none by 2.5 cc., by 3.0 cc. or by 4.0 cc. However, in one culture a considerable reduction in the number of larvae isolated was caused by the 4.0 cc.; 5.0 cc. of this solution sterilized two cultures, but 5,000 larvae were obtained from the third; 7.5 cc. and more killed the larvae with more regularity; 7.5 cc. contains the equivalent of 8.0% of the weight of the treated feces.

Used as a 1:4 solution, 5.0 cc. killed the larvae in one of the three cultures made, but the smallest number of larvae from a culture treated with 7.5 cc. was 4,800. Both 10.0 cc. and 12.5 cc. of a 1:4 solution were more or less effective in two of the three cultures treated by each, while 15.0 cc. was definitely effective; this quantity is equivalent to 8.75%.

A 1:8 solution was comparatively more effective. One culture was sterilized by 15.0 cc., two of three by 20.0 cc. while the third only contained

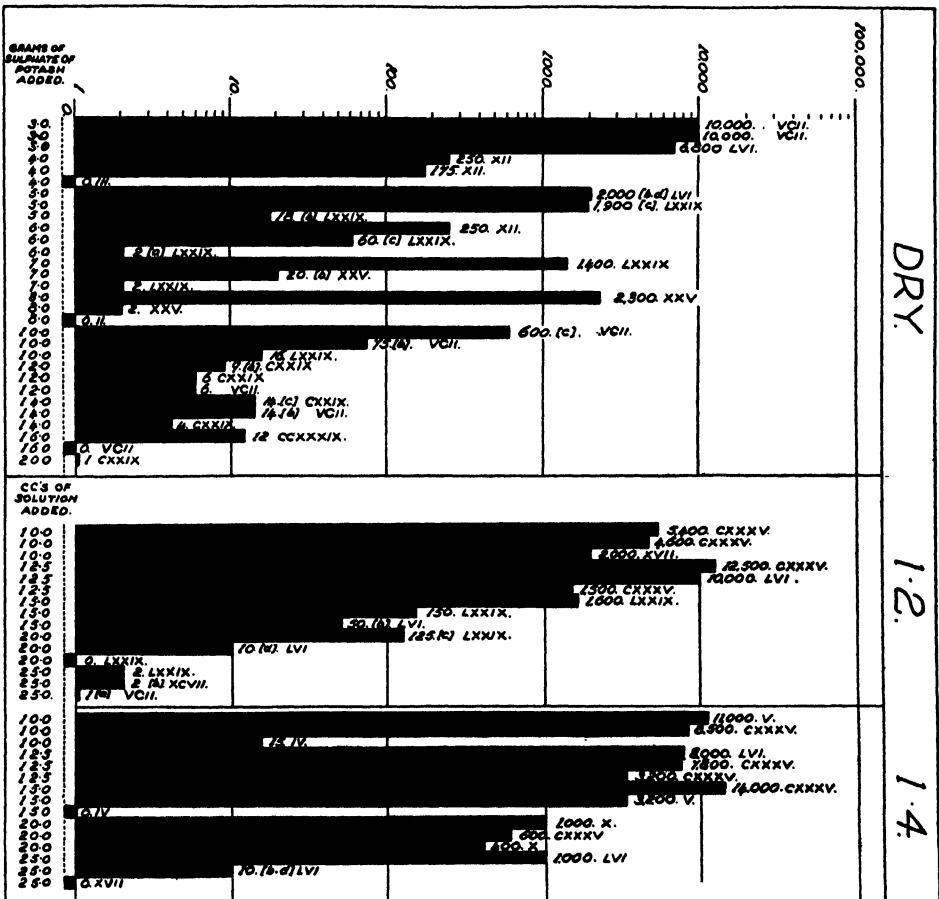


FIG. 5. Results obtained with sulphate of potash, dry and in solution.

150 larvae. All the cultures treated by 25.0 cc. were completely sterilized; 20.0 cc. of this solution contains 6.25%.

A 1:20 solution of this fertilizer was also ineffective; in only one of the three cultures treated with 25.0 cc. were most of the larvae killed.

These results suggest that on an average, potassium carbonate must be used at the rate of 7.7% of the weight of feces to produce sterilization against the free-living stages of sclerostomes.

### *Sulphate of Potash*

Sulphate of potash, which contains approximately 50% of potash, is the least lethal of the commoner potassic fertilizers, and it is extremely irregular in its action. It is therefore unlikely ever to make a practical sterilizing agent against sclerostomes under any conditions.

Dry quantities of between 0.5 and 20.0 gm. were tested. Fig. 5 shows that one culture was sterilized by 2.0 gm. and another by 4.0 gm. and that two others treated with the same amount yielded only a few larvae. However, many larvae were isolated from one culture treated with 7.0 gm. more from one treated with 8.0 gm. and a considerable number from one treated with 10.0 gm. Frequently some of the larvae were found dead when 5.0 gm. or more had been added.

Comparison of the cultures that were sterilized by 8.0 gm. or less with those that were not sterilized by an equal or greater amount, suggests that on an average 8.0 gm., or 20%, may be expected to cause sterilization. Under the conditions of these tests it is difficult to apply such large proportions of chemical in solution.

Complete sterilization was caused by 25.0 cc. of a 1:2 "solution," while 20.0 cc. was almost effective. The latter amount is equivalent to 21% of fertilizer.

Two out of three cultures were sterilized by 25.0 cc. of a 1:4 solution containing slightly over 14% of the weight of the treated feces. A reduction in the number of larvae isolated was brought about by 25 cc. of both 1:8 and 1:20 solutions.

### *Superphosphate*

#### PHOSPHATIC FERTILIZERS

Superphosphate is produced when natural phosphate rock is treated with sulphuric acid (25). The sulphuric acid unites with the lime displaced and forms sulphate of lime or gypsum, which comprises 40%-50% of the fertilizer. Two types of superphosphate have been tested, containing respectively 16% and 20% of available phosphate. Superphosphate can be mixed with manure without causing loss of ammonia.

Quantities of 0.5 gm. to 20.0 gm. of 20% superphosphate were mixed with 40 gm. of fresh feces. All the cultures treated with 8.0 gm. (20%) and more were sterilized, while lesser quantities caused a reduction in the number of the larvae and the death of some of those which were recovered.

Fig. 6 illustrates the results obtained with this fertilizer. The number of larvae that reached the infective stage and subsequently died suggests that superphosphate contains a slow-acting poison or is extremely local in its action, killing the larvae only when they happen to migrate into it. However, since such large quantities of carefully mixed material have been applied, the former explanation is the more likely one.

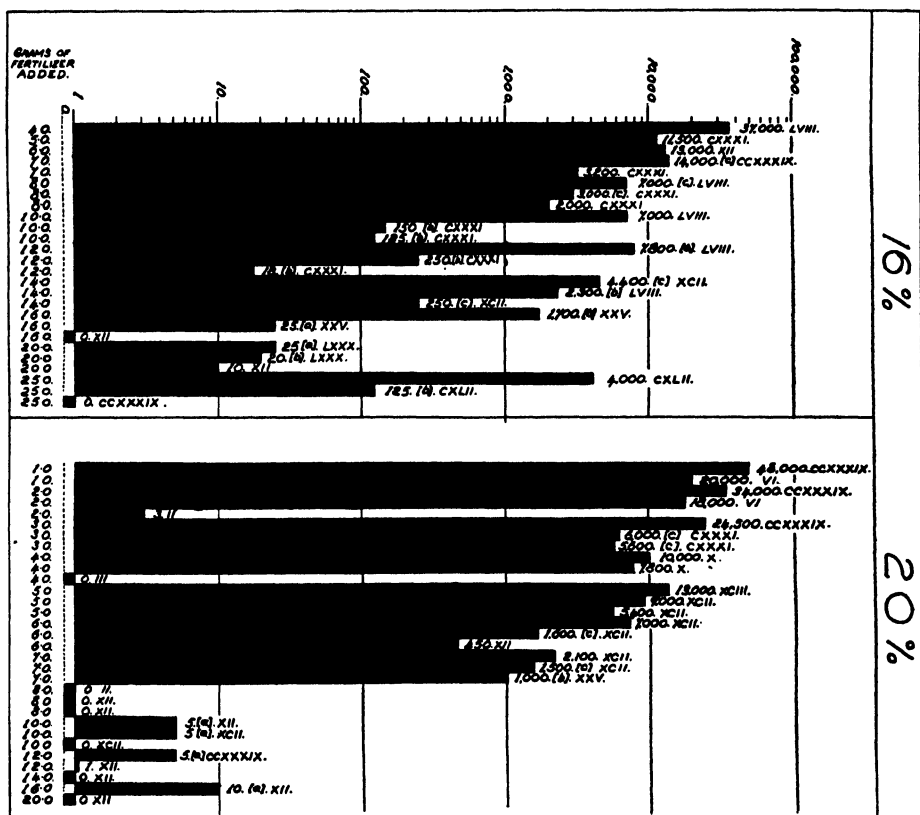


FIG. 6. Results obtained with 16% superphosphate and 20% superphosphate, both dry.

Comparison of the effectively sterilized cultures with those in which the larvae escaped, suggests that sterilization may be effected by 16% or 20% superphosphate, but a double quantity must be applied if 16% material is used. (See Fig. 6). Therefore, 16% superphosphate would have to be used at the rate of two of fertilizer to five of fresh feces, a proportion which is too great to be practical.

Basic slag and raw rock phosphate were also tested dry in quantities of 1.0 to 25.0 gm. None of the cultures suggested that either fertilizer has any value as a sterilizing agent against the free-living stages of sclerostomes in feces.

## LIME

Lime has been tested in three forms: ground limestone, quicklime and hydrated lime. Burning 3,571 lb. of limestone produces 2,000 lb. of quicklime which, in turn, yields 2,643 lb. of freshly slaked lime (25). Lime is unsuitable for mixing with manure, as it drives off ammonia. All three forms have been tested dry in quantities of 1.0 to 25.0 gm.

Ground limestone (carbonate of lime) showed no measurable lethal value. Quicklime (caustic lime) is peculiar because of the fact that even in cultures treated with 25.0 gm., many larvae reached the infective stage, although all larvae in the cultures treated with 20.0 and 25.0 gm., and many in those treated with 16.0 gm., and even considerably less, subsequently died. When larger quantities of feces and quicklime are mixed, the free-living stages are,

TABLE I  
CONTROLS FOR CULTURES TABULATED IN FIGS. 1-6

Series number	Date cultures made	Days kept in C.T. room	Average number of larvae isolated
ii	August 27, 1934	10	23,000
iii	August 27, "	17	25,000
iv	September 19, "	11	1,100
v	October 5, "	20	7,600
vi	December 12, "	19	21,000
vii	January 4, 1935	11	21,000
viii	January 4, "	24	21,000
ix	February 13, "	31	44,000
x	March 25, "	23	17,000
xi	April 3, "	10	41,000
xii	April 23, "	30	42,000
xvii	May 25, "	49	12,000
xxv	July 9, "	16	70,000
xxvii	July 11, "	16	9,000
lvi	December 13, "	21	58,000
lvii	December 13, "	24	15,500
lviii	December 17, "	20	31,000
lxxix	February 5, 1936	26	25,000
lxxx	February 5, "	27	19,500
lxxxii	February 13, "	25	28,000
xcii	March 11, "	22	26,000
xciii	March 19, "	14	27,000
vcii	March 20, "	35	26,000
cxxix	June 11, "	16	21,000
cxxx	June 11, "	18	14,000
cxxxii	June 11, "	22	14,500
cxxxiii	June 12, "	22	8,500
cxxxv	June 12, "	27	4,800
cxlii	June 23, "	17	12,500
clxxiv	July 17, "	13	8,500
clxxv	November 27, "	59	65,000
ccvi	November 27, "	60	76,000
ccix	February 12, 1937	52	36,000
ccxxxviii	February 18, "	46	40,000
ccxxxix	April 13, "	23	62,000
ccxl	April 15, "	25	62,000
ccxli	May 11, "	20	22,000
ccxlii	May 18, "	27	49,000
ccxliiv	May 20, "	25	49,000

of course, killed by the heat of the chemical reaction, which can be sufficient to char the feces.

Hydrated lime (slaked lime) in quantities of at least 20 and 25 gm., to 40 gm. of feces, killed many, but not all, of the numerous larvae which reached the infective stage. Hydrated lime was also tested in aqueous "solutions."

Twenty-six cultures were treated with as much as 25 cc. of various strengths (1:4 to 1:50). But they failed completely to give any indication that hydrated lime in solution is of any value as a sterilizing agent against sclerostomes in feces. This does not mean, of course, that lime used as a wash on stable walls, etc., will not kill the larvae there either chemically or by imprisoning them.

In addition to the cultures already discussed a few were made with muriate of potash, 16% superphosphate, hydrated lime and ground limestone, in which straw was incorporated. In this series, when the fertilizers were mixed dry, water was subsequently added. The eggs in this series were in a more advanced stage when the fertilizers were added. Muriate of potash appeared to be slightly less lethal when used in this way, while hydrated lime showed more satisfactory qualities than before. However, sufficient numbers of cultures have not been made to give the slight differences any significance.

### Conclusion

All the non-nitrogenous fertilizers have a low lethal value when used to control the free-living stages of sclerostomes in fresh manure, but because only the outside of a well built heap of horse manure has to be treated to make the manure safe for spreading on fields which are to be grazed by horses in a few years, a few non-nitrogenous fertilizers such as kainit and even 20% superphosphate may occasionally be used for sterilizing fresh feces. The other advantages which these fertilizers possess should encourage their use.

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## INVESTIGATIONS ON TRICHINOSIS IN CANADA

### I. A PRELIMINARY SURVEY OF THE INCIDENCE OF *TRICHINELLA SPIRALIS* IN HOGS IN EASTERN CANADA<sup>1</sup>

By THOMAS W. M. CAMERON<sup>2</sup>

#### Abstract

In a preliminary survey during 1937, 729 hogs from eastern Canada were examined by both digestion and compression techniques. Fifteen or 2.06% were found to be infected with living encysted larvae of *Trichinella spiralis*.

Recent investigations in the United States on the incidence of the larvae of the nematode worm *Trichinella spiralis* in the muscles of persons dead from various conditions, has shown a remarkably high incidence. Hall and Collins (1), who not only have conducted a large series of examinations at Washington, D.C., but have examined the results of other investigations, estimate that more than 12.5% of the inhabitants of the United States harbor this parasite.

There are no comparable figures for Canada. In 1901 Williams (2), examined a number of cadavers at Buffalo. Some were of Canadian origin and 16.6% of these were infected. Modern technique would probably have given a higher percentage of infection, but as they are, the figures are close to Hall and Collins' estimate for the United States. Since the recent interest in trichinosis, numerous clinical cases have been diagnosed in eastern Canada, and even though accurate statistics are not available, there is no doubt that the parasite is a common and important species here.

From 1898 until 1906 all hog carcasses intended for export to Germany and certain other countries in western Europe were examined in the United States for the presence of this parasite. In all, more than eight million carcasses were thus examined; of these, 1.41% contained *live* trichina larvae, while a further 1.16% contained trichina-like bodies. Since 1906 no regular inspections have been made, but in recent years some thousands of carcasses have been examined by the Bureau of Animal Industry for the presence of this parasite, in an attempt to check the increase or decline. The last figures available vary from about 5% for hogs fed on garbage to less than 1% for hogs fed no garbage. The total figures suggest that there is very little change since 1906.

No examinations have ever been made in Canada on a large scale and few even on a small one. Osler, in 1883, however, found that 0.4% of a small number of western hogs were infected.

It was, accordingly, considered advisable, in view of the apparent high human incidence in Canada to make a systematic examination of an un-

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selected series of hogs for the presence of this worm. The co-operation of the Health of Animals Branch of the Dominion Department of Agriculture was secured, and a letter was sent by the Veterinary Director General to six of the principal abattoirs in New Brunswick, Quebec, Ontario and Manitoba. It was realized that more than one year's work was involved in this investigation and, as a preliminary proceeding, only the six largest abattoirs within 24 hours of the Institute of Parasitology were selected. The officers-in-charge of each were asked to forward specimens from the diaphragms of hogs slaughtered in their abattoirs. They were asked to pick these at random but to choose them from pigs the source of origin of which was known. No selection was made on any other basis. Accordingly, each batch of specimens received consisted of several portions of pork, each from a different hog but all from the same district. It was found that packing in loosely covered glass containers permitted the arrival of material in good condition even in the warm weather of mid-summer. Wrapping in waxed paper and packing in cardboard, in most cases, was also suitable. In this way during the summer and early fall of 1937, 122 lots of material, containing in all 729 specimens of pork from the same number of Canadian hogs, were received in the laboratory.

These were examined both by digestion and by compression, while the last 94 specimens were examined by an iodine-stain technique as well.

For the digestion process, 10 gm. of muscle was used, chosen from areas of tendinous insertion where the sample made this possible. It was ground in a meat chopper and mixed with about 125 cc. of a 0.2% solution of pepsin in normal saline, maintained, by the addition of hydrochloric acid, at a pH of 1.0 to 2.0. This was digested for 24 hr. in the 38° C. constant-temperature room.

Owing to the cost of pepsin, during the course of the investigation the digestive enzyme was changed to papain. This proved not only cheaper but easier to use, the complete digestion making examination of the substrate easier and the wide pH at which papain acts removing the necessity for frequent

TABLE I  
SOURCES OF MATERIAL

	Hogs examined	Number infected
1. Canada Packers, Ltd., St. Boniface, Manitoba (Est. 7B).	276	5
2. Canada Packers, Ltd., Peterborough, Ontario (Est. 7F).	79	2
3. Canada Packers, Ltd., Montreal, Quebec (Est. 7D).	20	0
4. Wilsil's Ltd., Montreal, Quebec (Est. 25).	314	7
5. Eastern Abattoirs, Montreal, Quebec (Est. 22).	10	0
6. Swift Canadian Co. Ltd., Moncton, N.B. (Est. 18D),	30	1
	729	15

corrections. In 36 hr. at 38° C., 0.01 gm. of papain dissolved in 30 cc. of normal saline completely digested 10 gm. of pork, but actually the larvae were released much earlier.

Samples for examination by compression were examined in a Zeiss Trichina Compressorium under a binocular dissecting microscope. Towards the end of the series, a modification of the recent Kalwarijsky technique for the silver impregnation of Trichina larvae was also used. Thin portions of muscle were treated for 10 min. with a 0.5% solution of iodine in 1.0% potassium iodide. They were then washed in water and immersed in 5% sodium thiosulphate (hypo) solution until the muscle was free from the iodine color and became translucent. The larvae, however, retained the iodine and showed readily in the compressed translucent muscle as brown spiralled worms; the cysts themselves did not retain the color. However, no cases were detected by this technique which were not also seen in unstained material, although the use of iodine proved a valuable saving of time.

All the positive cases were found by both digestion and compression techniques except that only two of the three Ontario cases were seen in the compressorium.

Sources of material are given in Table I and the provinces of origin of the 15 hogs discovered to be infected are listed in Table II. The origin of the animals is given in more detailed form in Table III.

TABLE II  
ORIGIN OF INFECTED ANIMALS, BY PROVINCES

Province	Number examined	Positive
Saskatchewan	7	0
Manitoba	299	5
Ontario	146	3
Quebec	226	6
New Brunswick	40	0
Nova Scotia	5	0
Prince Edward Island	6	1
	729	15

TABLE III  
DETAILS OF ORIGIN OF INFECTED ANIMALS

	Hogs in infected lot	Number infected	Total portions received from district in 1937
<i>Manitoba</i>			
Rockwood Municipality	7	2	7
Cartier Municipality	10	3	80
<i>Ontario</i>			
Roseneath County	2	1	2
Lindsay County	2	1	4
Napanee County	7	1	9
<i>Quebec</i>			
Bagot	7	6	7
<i>Prince Edward Island</i>			
Prince County	6	1	6

### Discussion

This survey was of a preliminary nature and much detailed statistical information could not be expected from it. However, the percentage of infected hogs, out of 729 examined, was 2.06%, and this may be accepted as representing a probable rate of infection for eastern Canada, which is not too far from the true figure. Trichinosis is shown to exist in pigs in all the provinces from which large numbers of pigs were examined, as well as for Prince Edward Island, from which only six specimens were available. However, the provincial figures are too small to give any accurate figure for the prevalence of the parasite in any particular province. Although no trichinae were found in Saskatchewan, New Brunswick or Nova Scotia, it cannot be assumed that it is absent from these provinces as only 7, 40 and 5 specimens respectively, were received. On the contrary, it is probable that it does occur there also, although this cannot be declared with certainty until more material is examined.

The Canadian percentage differs only slightly from that obtained in the United States. The reasons for this are not apparent, as all garbage fed by commercial hog breeders in Canada must be cooked, whereas no such law applies in the United States. On the other hand, there is probably a higher percentage of hogs raised there on a purely corn diet.

Taking into account the fact that human trichinosis caused by eating flesh other than pork is negligible, the percentage of infected hogs found in this investigation is in agreement with the suggestion that the estimated rate of human trichinosis in the United States is probably also approximately correct for this country.

### Acknowledgments

I have to thank the Veterinary Director General of Canada and Dr. A. E. Cameron, the Chief Veterinary Inspector, for their generous co-operation in this investigation, and Drs. T. H. Bright, A. Cowan, E. Dufresne, A. S. Frame, E. Grandmaison, W. Kime, J. G. Macdonald, D. J. McLellan, J. A. McLeish, R. H. Rivington, and W. R. Wood, for their willing and expert assistance. Without it, the work would hardly have been possible. The actual examination of the samples in our laboratory was carried out under my supervision by Mr. L. L. Lyster, who was employed for this project with the aid of a special grant from the National Research Council of Canada.

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## THE FRESH-WATER MOLLUSCA OF SUB-ARCTIC CANADA<sup>1</sup>

BY ALAN MOZLEY<sup>2</sup>

### Abstract

A systematic account is given of the constitution, distribution and geographical affinities of the molluscan fauna occurring in fresh waters of sub-arctic Canada. The area covered is that part of western Ontario, Manitoba, Saskatchewan and Alberta lying north of N. Lat. 49°. A total of 111 species and varieties was collected and identified. Types of habitat available in this region for settlement by molluscs have been classified, and the local distribution of the species in them observed. A brief description of seven principal habitat types, with comments on their fauna in other parts of the sub-arctic region, is followed by a series of examples from specific localities. These molluscan associations, while not necessarily typical, are believed to be representative. The study is concluded with a comparison of the molluscan fauna of northern North America with that of northern Eurasia.

Three geographical elements in Canadian sub-arctic Mollusca are: a group of circumboreal species, a large number of strictly North American species, and a group characteristic of this region. An explanation of the close relation between the sub-arctic molluscs and those of the Mississippi drainage probably lies in the geological history of the region. There appears to have been a greater degree of speciation in North America than in northern Asia; the total number of species and varieties in sub-arctic Canada is 111, in northern Asia it is only 50. The explanation may lie partly in the richer source of supply, the greater facility for migration, and the wider range of habitats available in Canada. Thus new species as they arose would find suitable unoccupied habitats more readily. While this is hardly the sole explanation, the existence of some connection between physiography and speciation appears to be reasonable.

The following account deals with the constitution, distribution, and geographical affinities of the molluscan fauna of fresh waters in sub-arctic Canada. In so far as this report is based upon original observations in the field, it applies to the western part of the Province of Ontario, and to Manitoba, Saskatchewan and Alberta. Field work was carried on in that area from 1924 to 1931. During these and subsequent years the collections in several museums were studied, particularly those of the United States National Museum, and the British Museum (Natural History). This has made it possible to extend the scope of the work to include the whole of sub-arctic Canada. That is, it covers the greater part of the country lying to the north of N. Lat. 49°. Much of this vast territory remains to be explored, so that it is not possible to claim any degree of completeness in this work. Nevertheless sufficient is known about the mollusca of this region to justify an attempt to evaluate the fauna as a whole for purposes of comparison with other parts of the sub-arctic region.

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The first published account of the molluscs of this region, by G. M. Dawson, appeared in 1875. Other faunal lists, by Whiteaves (37, 38), Christy (11), and Hanham (16), followed. A general revision of the knowledge of the constitution of this fauna was undertaken by Dall during the early years of the present century, and was published in 1905. Since that time numerous short papers dealing with this fauna have appeared, and there has been a great advance in the study as a whole. A revision and extension of Dall's work is therefore needed.

### Systematic Account

This part of the paper consists of a list of the species and varieties which have been examined by the author and found to be distinct from all other members of the fauna. Definite localities are given, and also in most instances a concise summary of the geographical range of the diverse forms. A great effort has been made to avoid errors in the identification of the species. During the early part of the work very valuable assistance was rendered by several North American malacologists in the determination of specimens. In this connection thanks are due particularly to Mr. F. C. Baker, and to Dr. Bryant Walker. The late Dr. Victor Sterki was kind enough to examine the Sphaeriidae. Whatever changes may be made in the nomenclature of the group in the future, each of the forms included in this list should be recognizable, as each name is known to represent an actual animal which exists in nature in some numbers.

#### OUTLINE OF THE CLASSIFICATION OF THE FRESH-WATER MOLLUSCA OF SUB-ARCTIC CANADA

	Number of species and varieties		Number of species and varieties
GASTROPODA		PELECYPODA	
Family Lymnaeidae		Family Sphaeriidae	
<i>Lymnaea</i>	26	<i>Sphaerium</i>	12
Family Planorbidae		<i>Musculium</i>	5
<i>Planorbis</i>	17	<i>Pisidium</i>	15
<i>Planorbula</i>	3	Family Unionidae	
Family Physidae		<i>Quadrula</i>	1
<i>Physa</i>	4	<i>Amblema</i>	1
<i>Aplexa</i>	1	<i>Fusconaia</i>	1
Family Ancyliidae		<i>Strophitus</i>	1
<i>Ancylus</i>	1	<i>Anodonta</i>	3
<i>Ferrissia</i>	2	<i>Anodontoides</i>	1
Family Viviparidae		<i>Lasmigona</i>	2
<i>Campeloma</i>	1	<i>Proptera</i>	1
Family Valvatidae		<i>Actinonaias</i>	1
<i>Valvata</i>	3	<i>Ligumia</i>	1
Family Amnicolidae		<i>Lampsilis</i>	3
<i>Amnicola</i>	5		
Total Gastropoda	63	Total Pelecypoda	48

Total fresh-water mollusca

In addition to the forms that have been collected and examined in the course of this investigation and are included in the list, there are a number of records of other species and varieties in the literature. In the absence of specimens it appears best to leave these out of consideration for the time being. There is little doubt however, that the records and descriptions of Canadian mollusca published within recent years by Mr. F. C. Baker are to be relied upon, so in instances in which the author has not had the opportunity of examining adequate series of these forms, the names are listed at the end of the respective families. This critical policy may have resulted in certain valid species and varieties being ignored in the present paper, but it has the advantage of providing a firm basis for the further study of this interesting group of animals.

### Class GASTROPODA

#### FAMILY LYMNAEIDAE

Genus *Lymnaea* s.s. Lamarck 1799

*Lymnaea stagnalis jugularis* Say

*Lymnaea jugularis* Say, Art. Conchology, Nicholson's Encyc. I. 1817.

*Geographical Range.* *L. stagnalis* is found in Europe, northern Africa, northern Asia, and North America. The variety *jugularis* is confined to North America, east and west of the Rocky Mountains, from California and New York to Alaska.

Northernmost record: Lake Harrison, N. Lat. 70°.

Southernmost record: N. Lat. 37° in Colorado, 41° in Illinois and Ohio.

*Local Distribution in Canada.* This is one of the commonest fresh-water molluscs in the western part of Canada, and is included in almost every list of species from this region. Only new records are included in the list given below. The previously reported localities are summarized by Dall (13), and others may be found in the more recent papers listed in the bibliography. New records: Ontario—Oba River; Caramat; creek running into northern end of Long Lac; Nakina; Savant Lake district, Elbow Lake.

Manitoba—Lake Winnipegosis; marsh near the Meadow Portage; Grand Rapids of the Saskatchewan; Atikameg Lake, Mile 17, Hudson Bay Railway; Cormorant Lake, Mile 32, Hudson Bay Railway; Resting Lake, Mile 130; lake near Mile 137; creek at Mile 180.6; creek near Wintering Lake, Mile 186; lake near Mile 237; Split Lake; lake near Mile 286.

Alberta—Chestermere Lake, Calgary; Hastings Lake, Tofield; Hoople Lake, Entwistle; lake near Lac la Biche.

North West Territories—Mackenzie River delta.

Previous records published since Dall (13): Iglukitaktok, Mackenzie River delta, Dall (14). Southwest side of Mackenzie River, 30 miles above Fort Providence; at mouth of Hay River, Whittaker (39). Minaki, Ont., Winnipeg River, Mozley (21). Indian Bay station, Man.; Sturgeon Creek, near Winnipeg; St. Norbert, LaSalle River; Lockport; Clandeboyè, Muckle Creek;

Grand Beach, the Grand Marais; Jackfish Creek near Jackfish Lake; Victoria Beach; Treesbank; Souris River; Baldur, slough near Cobbs Lake; Ninette, marsh at northern end of Pelican Lake, slough 5 miles north of Ninette; Douglas Lake near Onah; Theodore, Sask., Powells Lake; Ingebright, Mozley (23). Mud Turtle Lake, near Brereton, Man., Mozley (22). Whiteshell River district, Man., Little Whiteshell Lake, Crow Duck Lake, Whiteshell River above Jessica Lake; Molson; Beausejour; Portage la Prairie; Macdonald; Delta; Ninette, Bone Lake; Basswood, Long Lake; Clear Lake, Riding Mountains; Dauphin; Lake Winnipegosis, marsh near the Meadow Portage; Kuroki, Sask., Fishing Lake; Humboldt, Burton Lake; Yonker, Eyehill Creek, Mozley (24). Birtle, Man., marshes along the course of Birdtail Creek, also in many ponds and small lakes to the north of Birtle; Kamsack, Sask., pond one-half mile west of Kamsack, also in Madge Lake, 20 miles north of the town; Margo, Margo Lake; Kuroki, lake 1 mile west of Whitesand Lake; Wadena, Foam Lake; Paskwegin, pond near Paskwegin Brook, also in small lake 2 miles south of Paskwegin siding; Elfros, Brich Creek; Quill Lake station, Quill Creek; Watson, Ironspring Creek; small lakes between Kelliher, Touchwood, and Hudson Bay Lake; Dalesford, creek flowing into Lake Lenore 2 miles south of the hamlet of Dalesford, Mozley (25). Marchington River, Sioux Lookout district, Ont., Baker and Cahn (9). 2 miles west of Invermay, Sask.; north shore of Fishing Lake, near Wadena; 9 miles northeast of Goodeve; Sandy Creek waterworks, near Moose Jaw, Russell (36). Kennedy, Sask.; small lakes in the Moose Mountain Forest Reserve; Shonts, Alta., Amisk Creek; Tofield, Beaverhills Lake; Wabamun, Lake Wabamun, also in the marsh between Wabamun and Kapasiwin; Junkins, Chip Lake, Mozley (30). Fossil deposit on the shore of the Pasquaia River, near The Pas, Man., Mozley (31). Freshwater Lake, Charlton Island, James Bay, Richards (35).

*Habitat.* Ponds which contain water permanently, small lakes, marshes on the shores of larger bodies of water, also in small marshes along the borders of sluggish streams.

*Lymnaea stagnalis jugularis* occurs most abundantly in the prairie and parkland regions. It is not particularly common in the forested region, and has little toleration of saline conditions. It has not been collected in any temporary ponds.

*Lymnaea stagnalis sanctaemariae* Walker

*Limnaea stagnalis sanctaemariae* Walker, Nautilus, 6 : 31. 1892.

*Geographical Range.* North America, from the Great Lakes region northwest to Manitoba.

Northernmost record: Wekusko Lake, N. Lat. 54° 50' (see below).

Southernmost record: Lake Michigan in N. Lat. 45°.

*Local Distribution.* New record: Wekusko (Herb) Lake, 10 miles west of Mile 81, Hudson Bay Railway, Man. Previous record: Lake Brereton, Man., Mozley (22).

*Habitat.* In large lakes, on smooth rocky shores sloping steeply into deep water in situations subject to severe wave action. In calm weather the

snails are to be found near the surface, but during storms they retire to the deeper water. The young individuals live in slightly more sheltered situations than the adults. The eggs of this variety, together with mature individuals, have been found in rock pools formed by the breaking of the waves on the shores, but this is probably not the usual site of egg-laying.

*Lymnaea stagnalis lillianae* Baker

*Lymnaea stagnalis lillianae* Baker.

*Geographical Range.* North America, from New York west to Manitoba. Northernmost record: Shoal Lake, Manitoba, N. Lat. 49° 30' (see below).

Southernmost record: Wisconsin, N. Lat. 46°.

*Local Distribution.* Previous records: Indian Bay, Shoal Lake, eastern Man., Mozley (24); Hill, Blackstone, St. Joseph, Bamaji, Fitchie, Abram, and Cat Lakes, Bamaji Lake outlet, Sioux Lookout district, Ont., Baker and Cahn (9).

*Habitat.* This variety has been found only on a sandy shore in Shoal Lake, where it was exposed to severe wave action.

*Lymnaea stagnalis wasatchensis* (Hemphill) Baker

*Lymnaea stagnalis wasatchensis* Baker, Chicago Acad. Sci., Spec. Pub. No. 3, 152. 1911.

*Geographical Range.* North America, confined to the western mountainous region.

Northernmost record: near Fort Anderson, N. Lat. 68°.

Southernmost record: Utah.

*Local Distribution.* Previous records: numerous localities in Jasper Park, N. Lat. 53°, Mozley (26, 30).

*Habitat.* The habitat of this variety in Jasper Park is usually among rooted aquatic plants in small lakes. It is occasionally found in larger bodies of water at relatively low altitudes (3000 to 4000 ft.).

*Lymnaea (Pseudosuccinea) columella casta* (Lea)

*Lymnaea-casta* Lea, Proc. Am. Phil. Soc. 2 : 33. 1841.

*Geographical Range.* North America, Ohio and Illinois north to Nova Scotia and Manitoba.

Northernmost record: Lake Brereton, Man. N. Lat. 49° 50'.

Southernmost record: *L. casta*, Ohio. *L. columella*, Florida, N. Lat. 27°.

*Local Distribution.* Previous record: Lake Brereton, eastern Man.

*Habitat.* In Lake Brereton this species was found on rocky shores in shallow bays where it was somewhat protected from wave action.

*Remarks.* Dall (13) records *L. columella* from Lake Winnipeg, and it may be that his record is based upon specimens of the variety *casta*.

*Lymnaea (Bulimnea) megasoma* (Say)

*Lymnaeus megasoma* Say, Rept. Longs Exped. 2 : 263. 1824.

*Geographical Range.* North America, from Vermont to Manitoba.

Northernmost record: Echimamish Lake district, Man., N. Lat. 57°.

Southernmost record: Stark Co., Ohio, N. Lat. 41°.

**Local Distribution.** New records: creek running into the northern end of Long Lac; Savant Lake district, Elbow and Heathcote Lakes, small lake south of Elbow Lake; Allenwater, Ont.

Previous records: Indian Bay station, Man., Falcon Bay, Mozley (21). Lake Brereton, Mozley (22). Whiteshell River district, shore of Whiteshell River below the unnamed lake 10 miles below Jessica Lake; quiet bay below third rapid below Betula Lake; near the eighth rapid below Betula Lake, Mozley (24). Rennie, Rennie River, Mozley (25). Marchington River; Bamaji Lake; Cat and Hill Lakes, Baker and Cahn (9).

**Habitat.** Lakes, ponds, and streams. Commonly but not invariably found in situations which are free from wave action. On shores and lake bottoms composed of rock, sand, and mud, and also among aquatic plants and in marshes. In favorable situations it may occur in large numbers.

*Lymnaea (Stagnicola) caperata* (Say)

*Lymnaeus caperatus* Say, New Harmony Diss. 2 : 230. 1829.

**Geographical Range.** North America, from Maryland, Colorado and California north to the mouth of the Mackenzie River.

Northernmost record: 30 miles north of Aklavik, N. Lat. 68° (approx.).

Southernmost record: Colorado.

**Local Distribution:** New records: Calgary, Alta.; 30 miles north of Aklavik, N.W.T.

Previous records: Molson, Man.; Navin; Sifton; Lake Winnipegosis, marsh near the Meadow Portage, Mozley (24). Fort Frances, Ont.; Grande Pointe, Man.; Birtle; Kamsack, Sask.; Elfros, Brich Creek; Kuroki, Van Patten Creek; Clair, Clair Brook; Quill Lake station, Quill Creek; Watson, Ironspring Creek; Lanigan; Kelliher; Touchwood; Dalesford, creeks flowing into Lake Lenore, Mozley (25). Wainwright, Alta., Viking; Kinsella; Irma; Shonts; Tofield; ponds near Cooking Lake; Lindbrook; Edmonton; Junkins, Chip Lake; Peace River district, 1 mile east of Faust; also along the shores of Lesser Slave Lake near Faust; Wanham, pond in the Birch Hills; creek entering Cadotte Lake from the south; 3 miles north of Spirit River, Mozley (30).

**Habitat.** Usually in temporary ponds, but sometimes to be found in marshes. This is a very common species on the prairies and parkland. It is somewhat less abundant in the forested region.

*Lymnaea (Fossaria) umbilicata* (C. B. Adams)

*Limnaea umbilicata* C. B. Adams, Am. J. Sci. 39 : 374. 1840.

**Geographical Range.** North America, New Jersey and Maine west to Manitoba.

Northernmost record: Birtle, Man., N. Lat. 50° 25'.

Southernmost record: Burlington, New Jersey.

**Local Distribution.** The only known occurrence of this species in the western part of Canada is at Birtle.

**Habitat.** The specimens were collected in a small marsh along the edge of Birdtail Creek.

**Remarks.** Mr. F. C. Baker identified the shells upon which this record is based.

*Lymnaea (Fossaria) parva sterkii* Baker

*Lymnaea sterkii* Baker, Nautilus, 19 : 51. 1905.

**Geographical Range.** North America, New York to Minnesota, Manitoba, and Alberta. *L. parva* f.t.—"James Bay . . . . . Maryland . . . . . Arizona", Baker (1).

Northernmost record: Jasper Park, Alta., N. Lat. 53°.

Southernmost record: Hickman Co., Tennessee.

**Local Distribution.** Previous records: Birtle, Man.; Jacques Lake district, tributaries of the Rocky River, Jasper Park, Alta.

**Habitat.** In both the above localities this snail was found in marshy areas along the border of streams. It appears to prefer muddy flats on which there is little vegetation.

*Lymnaea (Fossaria) dalli* Baker

*Lymnaea dalli* Baker, Bull. Ill. State Lab. Nat. Hist. 7 : 104. 1906.

**Geographical Range.** North America, from Kansas and Arizona through Ohio and Montana to Manitoba and Alberta.

Northernmost record: Pyramid Lake, Jasper Park, Alta., N. Lat. 53°.

Southernmost record: southern Arizona, N. Lat. 32° (approx.).

**Local Distribution.** Manitoba, Saskatchewan, and Alberta.

**Habitat.** Marshes and small lakes, usually among vegetation. This species has not been found in ponds, and appears to have little ability to withstand saline conditions.

*Lymnaea (Fossaria) modicella* (Say)

*Lymnaeus modicellus* Say, J. Phil. Acad. 5 : 122. 1825.

**Geographical Range.** North America, Texas to Nova Scotia and California, north to Manitoba.

Northernmost record: Ninette, Man., N. Lat. 49° 25'.

Southernmost record: Texas.

**Local Distribution.** Previous record: near Round Lake, Ninette.

**Habitat.** The habitat in the above locality is a small mud flat.

*Lymnaea (Fossaria) obrussa decampi* (Streng)

*Limnaea desidiosa* var. *decampi* Streng, Nautilus, 9 : 123. 1906.

**Geographical Range.** North America, Maine to Illinois, Manitoba, and Alberta.

Northernmost record: near Fort Providence, Mackenzie River, N. Lat. 61°.

Southernmost record: Illinois.

**Local Distribution.** New record: lake near Mile 137, Hudson Bay Railway. Previous records: Southwest side of Mackenzie River, 30 miles above Fort Providence, Mackenzie River district (as above); Mills Lake at mouth of Horn River, Whittaker (39). Clearwater (Atikameg) Lake, Mile 17, Hudson Bay Railway, Mozley (24). Bamaji, Hamilton, Cat, Pashkokogan Lakes, and

outlet of Kapikik Lake, Ont., Baker and Cahn (9). Postglacial fossil deposit near Lavenham, Man., Mozley (31).

*Habitat.* Lakes of moderate or large size, usually those which have clear and cold waters.

*Lymnaea (Fossaria) exigua* (Lea)

*Lymnaea exigua* Lea, Proc. Am. Phil. Soc. 2 : 33. 1841.

*Geographical Range.* North America, Tennessee north to Maine and Manitoba.

Northernmost record: creek running into the Grand Marais, at a point two miles east of Balsam Bay, Man., N. Lat. 50° 20'.

Southernmost record: Tennessee.

*Local Distribution.* Previous records: Mile 69, G.W.W.D.Ry., Man., Mozley (21). Onion Lake, near Minaki, Ont.; Clandeboye, Man., Mozley (24). Bamaji Lake outlet, Sioux Lookout district, Ont., Baker and Cahn (9).

*Habitat.* Usually in small marshy streams, or on the protected shores of small lakes.

*Lymnaea (Stagnicola) palustris* (Müller)

*Buccinum palustre* Müller, Verm. Terr. 2 : 131. 1774.

*Geographical Range.* Europe, northern Africa, northern Asia, North America. In North America from New Jersey, Arizona and California, north to James Bay, and Alaska.

Northernmost record: lake at N. Lat. 68° 20', W. Long. 141°.

Southernmost record: Arizona.

*Local Distribution.* New records: Fishing River station; Barrows Junction; Clear Lake, Riding Mountain; Miles 82 and 130, Hudson Bay Railway; small creek near Wintering Lake, Mile 186; ponds on the tundra north of Mile 510; tundra ponds near Esquimo Point, Man. Moose Jaw Creek, Sask. Deep Creek, 12 miles south of Entwistle; near Lac la Biche, Alta.

Previous records: Bernard Harbour, Arctic Coast, in part (?) described by Baker (6) as *Stagnicola kennicotti*, Dall (14); southwest side of Mackenzie River, 30 miles above Fort Providence, Mackenzie River district; south shore Second Lake, Horn River; Mills Lake at mouth of Horn River; at mouth of Hay River; south shore Great Slave Lake, near Sulphur Point, Whittaker (39). Mile 69, G.W.W.D.Ry., Man., Mozley (21). Indian Bay station, eastern Man.; Winnipeg, Catfish Creek; St. Vital; St. Norbert, La Salle River; Clandeboye, Muckle Creek; Goldeye Lake, near mouth of the Red River; Matlock; Balsam Bay, small pond in creek running into the Grand Marais, 2 miles east of Balsam Bay station; Jackfish Lake and Creek; Steep Rock; Aweme, slough near tamarack swamp; Ninette, marshy shores of Pelican Lake; Theodore, Sask., Whitesand River; Edmonton, Alta., Mozley (23). Cartier, Man., Stony Mountain; Portage la Prairie; Macdonald; Dauphin; Sifton; Molson; Delta, Mozley (24). Grande Pointe, Man., Kamsack, Sask.; Madge Lake, Duck Mountain; Kuroki; Paskwegin, Paskwegin Brook, also in Little Quill Lake; Clair, Clair Brook; Quill Creek; Wynyard; Watson, Ironspring Creek; Kelliher; Touchwood; Dalesford, Mozley (25).

Jasper Park, Alta., pond between Patricia and Katrine Lakes; Lake Mildred, Mozley (26). Wadena, Sask., north shore of Fishing Lake; Moose Jaw, Sandy Creek waterworks, Russell (36). Langbank, Sask.; Kennedy; small lakes in Moose Mountain Forest Reserve; Regina, Pile of Bones Creek; Bethune, near mouth of the Arm River; Wainwright, Alta.; Viking; Phillips; Kinsella; Irma; Shonts; Tofield, Beaverhills Lake, also in Amisk Creek; 3 miles southwest of Tofield; ponds near Cooking Lake; marsh between Wabamun and Kapasiwin; Junkins, Chip Lake; Peace River district, McLennan, Kimiwan Lake, Kinuso, Strawberry Creek near Lesser Slave Lake; Wanham, pond in the Birch Hills 4 miles south of Wanham, also in Cadotte (Ka Kut) Lake; 3 miles north of Spirit River; near White Mountain, Spirit River, Mozley (30). Agassiz clay near Fort Frances, Ont.; postglacial fossil deposit on the shore of the Pasquaia River near The Pas, Man., Mozley (31). Abitibi River, 12 miles north of Iroquois Falls, Ont., Richards (35).

This species has a very wide range in the western part of Canada, but is more common on the prairie and parkland than elsewhere.

*Habitat.* Temporary and permanent ponds, small and large lakes, streams. *L. palustris* thrives in temporary ponds in which there is water for only two months in each year. It occurs in large numbers in permanent ponds, small lakes, marshes, and usually in the more sluggish streams. It has also been found on a sandy shore of Lake Winnipeg in a position only slightly protected from wave action. It has a marked resistance to saline conditions, occurring in considerable numbers in parts of the Little Quill Lake, Sask. The waters of this lake were found to contain about 9700 parts per million of dissolved materials, chiefly sodium chloride, sodium sulphate, and magnesium sulphate (see Mozley, 25).

*Remarks.* *Lymnaea palustris* is an exceedingly variable species, and in the past different names have been applied to the variant forms. Since the range of variation is so great (see Mozley, 32), it appears best to adopt a single general name for the majority of the animals of this group. Subspecific names may be recognized later. Since large series of the species and varieties recently described by Baker (5-7) have not been available, it has been impossible to study them critically, and they are not included in this account. This does not necessarily mean that they are unrecognizable as distinct races, but merely that the writer has been unable to reach any conclusion regarding them.

*Lymnaea (Stagnicola) palustris castorensis* Mozley

*Lymnaea traskii castorensis* Mozley, Proc. Malac. Soc. London, 20 : 241. 1933.

*Geographical Range.* North America, a pond formed by beavers (*Castor canadensis*) near Medicine Lake, Maligne drainage, Jasper Park, Alta. This is the only known locality.

*Remarks.* In the original description this form was given as a variety of *L. traskii* Tryon. As that "species" appears to be merely a variant of the *palustris* group it may be preferable to treat *castorensis* as a variety of *palustris*.

*Lymnaea (Stagnicola) traskii* (Tryon)

*Limnaea traskii* Tryon, Proc. Phil. Acad. 149. 1863.

**Geographical Range.** North America, confined to the western mountainous region.

Northernmost record: Twin Lakes, Maligne drainage, Jasper Park, Alta.

Southernmost record: California.

**Local Distribution.** Banff, and numerous localities in Jasper Park. (Mozley, 26).

**Habitat.** This species is usually found on open shores of large lakes but has also been collected in ponds.

*Lymnaea (Stagnicola) saskatchewanensis* Mozley

*Lymnaea vahlii saskatchewanensis* Mozley, Am. Mid. Nat. 13 : 236. 1932.

**Geographical Range.** North America, interglacial deposit near Beaubier, Sask.

**Remarks.** Mr. F. C. Baker considers that this should be regarded as a distinct species.

*Lymnaea (Stagnicola) hedleyi* Baker

*Lymnaea hedleyi* Baker, Nautilus, 40 : 23. 1927.

**Geographical Range.** North America, the headwaters of the Fraser River, British Columbia.

**Local Distribution.** New record: Lucerne, B.C., Fraser River.

Previous record: Red Pass Junction, Fraser River (Baker, *loc. cit.*).

**Habitat.** This species was found in large numbers on stones on the bottom of the Fraser River in places where the current was strong, but the surface of the water unbroken.

**Remarks.** The new record extends the known distribution of this species upstream to within a few miles of the continental divide at the Yellowhead Pass.

*Lymnaea (Stagnicola) lanceata* (Gould)

*Limnaea lanceata* Gould, Proc. Boston Soc. Nat. Hist. 3 : 64. 1848.

**Geographical Range.** North America, Ohio and Wisconsin north to Ontario and Manitoba.

Northernmost record: Canyon Lake, Ont., N. Lat. 50° (approx.).

Southernmost record: Summit Co., Ohio, N. Lat. 41°.

**Local Distribution.** New record: Canyon Lake.

Previous records: Mile 95, G.W.W.D.Ry., Mozley (23); several localities on the Whiteshell River, Mozley (24); Lake Brereton, Man., Mozley (22).

**Habitat.** Marshes, particularly those near streams or large bodies of water.

*Lymnaea (Stagnicola) catascopium* Say

*Lymnaea catascopium* Say, Nich. Encyc., Amer. Ed. 2. 1817.

**Geographical Range.** North America, Maryland, Ohio, and Nova Scotia.

Northernmost record: Great Slave Lake, N. Lat. 61°.

Southernmost record: Maryland.

*Local Distribution.* Previous records: Lake Winnipeg, Mozley (23).

*Habitat.* Exposed shores of large lakes, particularly those which are strewn with large boulders.

*Lymnaea (Stagnicola) emarginata* (Say)

*Lymnaeus emarginatus* Say, J. Phil. Acad. 2 : 170. 1821.

*Geographical Range.* North America, from New York, Michigan and Ontario to Alberta.

Northernmost record: Grand Rapids of the Athabasca, N. Lat. 56° (approx.).

Southernmost record: New York State, N. Lat. 45°.

*Local Distribution.* New record: Grand Rapids of the Athabasca.

Previous records: Lake Winnipeg, Mozley (23); Lake Winnipegosis; Atikameg Lake, Mile 17, Hudson Bay Railway, Mozley (24).

*Habitat.* The shores of large lakes, particularly those which are subject to severe wave action.

*Lymnaea (Stagnicola) emarginata angulata* (Sowerby)

*Limnaea angulata* Sowerby, Conch. Icon. 18 : Lim. No. 47. 1872.

*Geographical Range.* North America, Michigan to Manitoba.

Northernmost record: Winnipeg River, Man., N. Lat. 50° 15'.

Southernmost record: Michigan.

*Local Distribution:* Previous record: Winnipeg River, near the mouth of the Whitemouth River, Mozley (21).

*Lymnaea (Stagnicola) emarginata canadensis* (Sowerby)

*Limnaea canadensis* Sowerby, Conch. Icon. 18 : Lim., sp. 45. 1872.

*Geographical Range.* North America, from New York north to Anticosti, and west through Michigan and Wisconsin to Manitoba and Alberta.

Northernmost record: Northern Twin Lake, Maligne drainage, Jasper Park, Alta., N. Lat. 53°.

Southernmost record: southern New York, Baker.

*Local Distribution.* Winnipeg River, near Minaki, Ont., Mozley (21); numerous localities in Jasper Park, Mozley (26).

*Lymnaea (Stagnicola) walkeriana* (Baker)

*Stagnicola walkeriana* Baker, Nautilus, 39 : 119. 1926.

*Geographical Range.* North America, Lakes Superior and Michigan, far western Ontario.

Northernmost record: Winnipeg River, N. Lat. 50°.

Southernmost record: Lake Michigan, near Sturgeon Bay.

*Local Distribution.* Previous records: English River, near its junction with the Winnipeg River; Lost Lake, near Minaki, Ont.

*Lymnaea preblei* Dall

*Lymnaea preblei* Dall, Harriman Alaska Exped. 13 : 70. 1905.

*Geographical Range.* North America, far western Ontario, northern Manitoba and Saskatchewan.

Northernmost record: Clear Lake, Sask., N. Lat. 56°.

Southernmost record: English River, Ont.

*Local Distribution.* Previous records: English River; Knee Lake, northern Man.; Lac Ile à la Crosse, and Clear Lake.

*Remarks.* This is a recognizable form which will probably prove to be closely related to *L. emarginata*. *L. binneyi* is omitted from this account as it appears difficult to recognize the limits of its variation, some of the named specimens approaching *L. emarginata* very closely.

*Lymnaea randolphi* Baker

*Lymnaea randolphi* Baker, Nautilus, 18 : 63. 1904.

*Geographical Range.* North America, Yukon Territory and Alaska.

Northernmost record: lake near Cosmos River, north of the Kowak River, Alaska, about N. Lat. 68°.

Southernmost record: Lake Lindeman, Y.T., N. Lat. 60°.

*Local Distribution.* Several localities in the Yukon Territory and Alaska.

*Remarks.* Dall (13, p. 71) and Baker (1, p. 453) cite a locality in the Lillooet district of British Columbia. The specimens in the United States National Museum from this locality are altogether distinct from *L. randolphi*. The locality from Washington given by Dall is also erroneous.

*Lymnaea atkaensis* (Dall)

*Lymnaea ovata* var *atkaensis* Dall, Proc. U.S. Nat. Mus. 7 : 343. 1884.

*Geographical Range.* Aleutian Islands, Behring Sea.

*Local Distribution.* Lake on the Island of Atka, Aleutian Chain, near Korovin Bay (Dall).

*Lymnaeidae Reported on Good Authority to Occur in Sub-arctic  
Canada, but not Examined by the Author*

*Lymnaea (Stagnicola) alberta* (Baker), Brazeau Lake, Alta., Baker (2). This species has not been recognized among the collections from Brazeau Lake made by the present author. Most of the specimens from this lake, and the valley below it as far north as Isaac Creek, were *L. traskii* or similar members of the palustris group.

*Lymnaea (Stagnicola) exilis* Lea, reported from several localities in the Sioux Lookout district by Baker and Cahn (9). None have been seen by the author. Specimens of *L. lanceata* (Gould) from Canadian localities have been compared and found to be identical with the type in the collection of the United States National Museum.

*Lymnaea (Stagnicola) catascopium kempfi* (Baker and Cahn (9)), reported from several localities in the Sioux Lookout district, Ont.

*Lymnaea (Stagnicola) palustris alpenensis* Baker, reported from Bamaji Lake, Sioux Lookout district, by Baker and Cahn (9).

*Lymnaea (Stagnicola) kennicotti* (Baker) (6), Bernard Harbour, Arctic Canada.

*Lymnaea (Stagnicola) palustris ungava* (Baker) (6), Fort Chimo, Ungava.

## FAMILY PLANORBIDAE

Genus *Planorbis* Geoffroy 1767*Planorbis (Helisoma) antrosus* Conrad

*Planorbis antrosus* Conrad, Am. J. Sci. 25 : 343. 1834.

*Geographical Range.* North America, from Alabama and Arkansas north to Massachusetts, Illinois and Manitoba.

Northernmost record: Birtle, Man., N. Lat. 50° 20'.

Southernmost record: Alabama.

*Local Distribution.* Previous record: Birtle, Mozley (23).

*Habitat.* These specimens were collected in Birdtail Creek, near Birtle.

*Remarks.* These shells were *P. antrosus* f.t., but some individuals approached the variety *portagensis* Baker.

*Planorbis (Helisoma) antrosus sayi* (Baker)

*Helisoma antrosa sayi* Baker, Bull. Wisc. Geol. & Nat. Hist. Surv. 70 : 322. 1928.

*Geographical Range.* North America, New York, the Great Lakes region, Ontario to Alberta.

Northernmost record: Knee Lake, northern Man., N. Lat. 55°.

Southernmost record: ? New York.

*Local Distribution.* New records: Knee Lake.

Previous records: Indian Bay station, Man., Falcon Bay; Mozley (23). Lake Brereton; Mud Turtle Lake, Mozley (22). Lake Winnipegosis; Mossy River near Winnipegosis, Mozley (24). Madge Lake, north of Kamsack, Sask., Mozley (25). Pyramid Lake, Jasper Park, Alta., Mozley (26). Wabamun, Alta., Lake Wabamun, Mozley (30). Postglacial fossil deposit, near Lavenham, Man., Mozley (31).

*Habitat.* Usually in small streams or those of moderate size.

*Planorbis (Helisoma) antrosus royalensis* Walker

*Planorbis bicarinatus royalensis* Walker, Nautilus, 23 : 9. 1909.

*Geographical Range.* North America, Isle Royale, Lake Superior; far western Ontario.

Northernmost record: Lac Seul district, Ont., N. Lat. 50° (approx.).

Southernmost record: Isle Royale, N. Lat. 48°.

*Local Distribution.* Previous records: Bamaji, St. Joseph, Hamilton, Cat and Botsford Lakes; outlet of Bamaji Lake, Ont., Baker and Cahn (9).

*Planorbis (Helisoma) trivolvis* Say

*Planorbis trivolvis* Say, Nich. Encyc., 1st ed., II, pl. ii, fig. 2. 1817.

*Geographical Range.* North America, far northeastern Asia. In North America, from Tennessee and Missouri north to Alaska.

Northernmost record: Fort Yukon, Alaska, N. Lat. 66° 40'.

Southernmost record: Tennessee.

**Local Distribution.** *P. trivolvis* has been reported from a great many localities in sub-arctic Canada. It is most abundant on the prairies, and least common in the forested region. There are shells in the collection of the United States National Museum from the Seward Peninsula, Alaska, labelled, "Under 12 ft. muck on cobbles." Baker (8) is of the opinion that many of the previous records of *P. trivolvis* actually relate to other species, but reports that the typical form is found as far north and west as Wainwright, Alta.

**Habitat.** *P. trivolvis* is usually found in marshy places along the borders of lakes or along the edges of streams. It has little ability to withstand saline conditions, but appears to be able to withstand desiccation to a very slightly greater extent than *Lymnaea stagnalis*.

*Planorbis (Helisoma) trivolvis pilsbryi* Baker

*Planorbis trivolvis pilsbryi* Baker, Nautilus, 39 : 117. 1926.

**Geographical Range.** North America, Massachusetts and New York west to Wisconsin, Ontario, and Manitoba.

Northernmost record: Landing River, northern Man., N. Lat. 56°.

Southernmost record: northern New York.

**Local Distribution.** New record: Landing River, second portage from the Hudson Bay Railway on the route to Split Lake.

Previous records: Indian Bay station, Man., Falcon Bay; Brereton, Lake Brereton, Mozley (25). Marchington River, Sioux Lookout district; Sturgeon Lake, Rainy River district, Baker and Cahn (9).

**Habitat.** In marshes, and on submerged vegetation in situations protected from wave action.

*Planorbis (Helisoma) corpulentus* Say

*Planorbis corpulentus* Say, Longs Exped. 2 : 262. 1824.

**Geographical Range.** North America, northern Michigan and Minnesota, Ontario, Manitoba, and Saskatchewan. This species is confined to the forested region.

Northernmost record: Lac Ile à la Crosse, Sask., N. Lat. 55° 45'.

Southernmost record: northern Minnesota, Baker.

**Local Distribution.** Numerous localities in far western Ontario, Mozley (21), Baker and Cahn (9). Also known to occur at Falcon Bay, Shoal Lake, eastern Man., Mozley (24).

**Habitat.** Lakes and streams. Usually in somewhat protected situations, but not in marshes.

*Planorbis (Helisoma) corpulentus multicostatus* (Baker)

*Helisoma corpulentum multicostatum* Baker, Nautilus, 46 : 7. 1932.

**Geographical Range.** North America, far western Ontario.

**Local Distribution.** Kahnipiminanikok Lake, Rainy Lake district; Cherry Island, Rainy Lake; Root River (McInnes coll.); Hill, Birch and St. Joseph Lakes, Kenora district; swamp of Lac des Mille Lacs, Sturgeon and Abram Lakes, Thunder Bay district, all in Ontario, Baker (8).

*Planorbis (Helisoma) infracarinatum* (Baker)

*Helisoma infracarinatum* Baker, Nautilus, 46 : 8. 1932.

*Geographical Range.* North America, Ontario, Manitoba, northern United States.

Northernmost record: Wekusko Lake, N. Lat. 54° 50'.

Southernmost record: "The United States," Baker.

*Local Distribution.* New records: Isle La Crosse, English River (*sic*), U.S.N.M. No. 29231, Kennicott coll., det. Baker; Wekusko (Herb) Lake, 10 miles west of Mile 81, Hudson Bay Railway.

Previous record: Basswood River rapids, Rainy River district, Ont., Baker (8).

*Planorbis (Helisoma) campanulatus wisconsinensis* Winslow

*Planorbis campanulatus wisconsinensis* Winslow, Occ. Papers Mus. Zool. Univ. Mich. 180 : 5. 1926.

*Geographical Range.* North America, Michigan and Quebec, west to Illinois, Ontario, and Manitoba. This variety is confined to the forested region. Northernmost record: Landing Lake, Hudson Bay Railway, N. Lat. 55° 20'. Southernmost record: Michigan.

*Local Distribution.* New record: Landing Lake.

Previous records: Indian Bay station, Man., Falcon Bay; Minaki, Ont., Winnipeg River; Star Lake near Redditt; Alice and Onion Lakes near Minaki; English River near its junction with the Winnipeg River, Mozley (21). Snake Lake, near Indian Bay station, Man., Mozley (23). Brereton and Mud Turtle Lakes, Mozley (22). Several small lakes on Duck Mountain near Madge Lake, north of Kamsack, Sask., Mozley (25).

*Habitat.* Small lakes, and also to some extent in those of moderate size. Frequently found on bare stones and rock faces, but not in the most exposed situations.

*Planorbis (Helisoma) campanulatus rudentis* Dall

*Planorbis campanulatus* var. *rudentis*, Dall, Harriman Alaska Exped. 13 : 90. 1905.

*Geographical Range.* North America, Michigan to Manitoba.

Northernmost record: Knee Lake, Man., N. Lat. 55°.

Southernmost record: Marl Lake, Roscommon Co., Michigan.

*Local Distribution.* Knee Lake.

*Planorbis (Helisoma) campanulatus davisi* Winslow

*Planorbis campanulatus davisi* Winslow, Occ. Papers Mus. Zool. Univ. Mich. 180 : 8. 1926.

*Geographical Range.* North America, Michigan to southern Manitoba.

Northernmost record: Douglas Lake, near Onah, Man., N. Lat. 49° 45'.

Southernmost record: Pinnebog River, Huron Co., Michigan.

*Local Distribution.* Previous record: Douglas Lake, Mozley (24).

*Planorbis (Menetus) exacuus* Say

*Planorbis exacuus* Say, J. Phil. Acad. 2 : 168. 1821.

*Geographical Range.* North America, east of the Rocky Mountains, from New Mexico north to Alaska.

Northernmost record: left bank of the Yukon River below Fort Yukon (Pleistocene marl), N. Lat. 66° 40'.

Southernmost record: New Mexico.

*Local Distribution.* Previous records: south shore Second Lake, Horn River; south shore Lake Kakisa, near mouth of Kakisa River, Mackenzie River district, Whittaker (39). Minaki, Ont., Winnipeg River, Mozley (21). Indian Bay station, Man., Falcon Bay, and Snake Lake; Grand Beach; Douglas Lake near Carberry, Mozley (23). Lake Brereton, Mozley (22). Whiteshell River district, Whiteshell Lake and vicinity; small lake on portage between Whiteshell and Crow Duck Lakes; Macdonald; Lake Winnipegosis, near the Meadow Portage, and on the lake bottom near Snake Island; Clearwater (Atikameg) Lake, Hudson Bay Railway, Mozley (24). Small lake one-half mile west of Madge Lake, north of Kamsack, Sask.; Wadena, Foam Lake; Kuroki, Van Patten Creek; Kelliher; Touchwood, Mozley (25). Hibernia Lake near Jasper, Alta., also in Marjorie, the larger Trefoil Lake, an unnamed lake north of Geikie station, Annette, Caledonia, Edna, and Pyramid Lakes, pond in the bed of Pyramid Creek, and other localities, Mozley (26). Small lakes in Moose Mountain Forest Reserve, Sask.; Phillips, Alta.; Tofield, Beaverhills Lake; Junkins, Chip Lake; Peace River district, creek 3 miles east of Driftpile; Wanham, Cadotte Lake, Mozley (30). Cat, Bamaji and Kimmewin Lakes, Sioux Lookout district, Ont., Baker and Cahn (9).

*Habitat.* Temporary ponds, small lakes, marshes, on the bottom of large lakes, e.g., Lake Winnipegosis.

*Planorbis (Menetus) exacuus* *megas* Dall

*Planorbis exacuus* var. *megas* Dall, Harriman Alaska Exped. 13 : 91. 1905.

*Geographical Range.* North America, described from Birtle, Man. Stated by Baker (3) to be a northern form found principally in Wisconsin, Michigan, and Minnesota.

*Local Distribution.* Cat, Bamaji, and Kimmewin Lakes, Ont., Baker and Cahn (9).

*Planorbis (Gyraulus) deflectus* Say

*Planorbis deflectus* Say, Rept. Longs Exped. 2 : 261. 1824.

*Geographical Range.* North America, Massachusetts west to Nebraska, and north to Manitoba.

Northernmost record: Victoria Beach, Man., N. Lat. 50° 40'.

Southernmost record: Nebraska.

*Local Distribution.* Previous records: Victoria Beach; Jackfish Lake, east of Balsam Bay, Man., Mozley; Clearwater Lake, Hudson Bay Railway, Mozley (24).

*Remarks.* Dall (13) records *P. deflectum* from Great Slave Lake and Alaska. The specimens upon which these records are based are in the U.S. National Museum and are not *deflectus*. Some shells of this species approach *P. acronicus* Férussac.

*Planorbis (Gyraulus) arcticus* "Beck" Möller

*Planorbis arcticus* (Beck Mss.) Möller, Index Moll. Groenl. p. 5. 1842.

*Geographical Range.* North America and Greenland.

Northernmost record: Greenland or Alaska.

Southernmost record: Illinois (fossil), Baker.

*Local Distribution.* Previous records: Mackenzie River district, south shore of Fawn and Second Lakes, Horn River; western end Lake Kakisa; south shore Lake Kakisa near mouth of the Kakisa River; south side of Mills Lake, Whittaker (39). Sioux Lookout district, Ont., Kimmewin, Bamaji, Cat, St. Joseph, Hamilton, Kapikik, Pashkokogan, and Fitchie Lakes, Baker and Cahn (9).

This is a common species in the territory extending from Ontario to the Mackenzie basin. In the past it has been mistaken for *P. parvus* Say.

*Planorbis (Gyraulus) umbilicatellus* Cockerell

*Planorbis umbilicatellus* Cockerell, Conch. Exchange, 2 : 68. 1887.

*Geographical Range.* North America, New Mexico north to Manitoba.

Northernmost record: Touchwood, Sask., N. Lat. 51° 30'.

Southernmost record: Mesilla, New Mexico, N. Lat. 32° 15'.

*Local Distribution.* Previous records: St. Vital, Man., Mozley (21). Brereton, Mozley (22). Grande Pointe; Birtle; Kelliher, Sask.; Touchwood, Mozley (25). Small lakes and ponds in Moose Mountain Forest Reserve; Wainwright, Alta.; Viking; Shonts; Tofield; ponds near Cooking Lake; Edmonton; Peace River district, 3 miles north of Spirit River, Mozley (30).

*Habitat.* *P. umbilicatellus* occurs only in temporary ponds. The explanation of the rarity of this species in the forested region lies in the fact that the small basins which form its sole habitat are quickly filled with vegetation in that region.

*Planorbis (Gyraulus) crista* (Linné)

*Nautilus crista* Linné, Syst. Nat., ed. 10 : 709. 1758.

*Geographical Range.* Europe, northern Africa, northern Asia, North America.

Northernmost record: Fort Yukon, Alaska, N. Lat. 66° 40'.

Southernmost record: Illinois, Baker.

*Local Distribution.* New records: Fort Yukon; Athabasca Delta, Alta.

Previous records: Virl Lake, near Jasper, Alta.; also in the smaller Trefoil Lake, and Lake No. 6, Mozley (26). Tofield, Alta., Mozley (30).

*Habitat.* Ponds and small lakes.

Genus *Planorbula* Haldeman 1842*Planorbula armigera* Say

*Planorbis armigerus* Say, J. Phil. Acad. 2 : 164. 1818.

**Geographical Range.** North America, Louisiana and Georgia, north to New England and Canada.

Northernmost record: ? Manitoba, see below.

Southernmost record: Louisiana.

**Local Distribution.** Previous records: at mouth of Hay River, Mackenzie River district, Whittaker (39). Grand Beach, Man., the Grand Marais; Ninette, northern end of Pelican Lake, Mozley (23). Sioux Lookout district, Ont., Bamaji, Cat, St. Joseph, Botsford, Hamilton, and Fitchie Lakes, Baker and Cahn (9).

**Remarks.** With the exception of the records mentioned above the reports of this species from Canada must be regarded as doubtful as they may refer to the more recently described *P. crassilabris*.

*Planorbula crassilabris* Walker

*Segmentina crassilabris* Walker, Nautilus, 20 : 122. 1907.

**Geographical Range.** North America, Michigan, Iowa, north to Canada.

Northernmost record: Balsam Bay, Man., N. Lat. 50° 30'.

**Local Distribution.** Previous records: Waugh (Indian Bay station) near mouth of the Falcon River, Man., Mozley (20). Arnaud; Balsam Bay, small pond in creek flowing into the Grand Marais, 2 miles east of Balsam Bay station, Mozley (23). Lake Brereton, Mozley (22). Grande Pointe, Mozley (25). Junkins, Alta., Chip Lake; marsh between Kapasiwin and Wabamun; Peace River district, Kinuso, Strawberry Creek near Lesser Slave Lake; pond near Mile 196.7, E.D. & B.C. Ry.; small creek near Faust, Mozley (30).

**Habitat.** Ponds, small lakes, and marshes.

*Planorbula campestris* Dawson

**Geographical Range.** North America, from South Dakota to the North West Territories.

Northernmost record: Mackenzie River, 30 miles above Fort Providence, N.W.T., N. Lat. 61°, Whittaker (39).

Southernmost record: Coteau Hills, 5 miles northeast of Clear Lake, South Dakota.

**Local Distribution.** Previous records: Mackenzie River district, south shore Fawn Lake, Horn River; southwest side of Mackenzie River, 30 miles above Fort Providence; Mills Lake at mouth of Horn River; south side Mills Lake, Whittaker (39). Beulah, Man., Wade's slough, Mozley (23). Wadena, Sask., Mozley (24). Birtle, Man., Kelliher, Sask.; Touchwood, near Hudson Bay Lake, Mozley (26). Ponds in Moose Mountain Forest Reserve; Phillips, Alta.; Viking; Tofield; Peace River district, 3 miles north of Spirit River.

This species has not yet been found in the densely forested region by the author. The precise habitat is not known of the specimens upon which the

records from the Mackenzie River district are based, but in that region this species may be confined to "islands" of grassland in the forest.

*Habitat.* Temporary ponds, rarely in permanent ponds which have a temporarily flooded area surrounding them.

*Planorbidae Reported on Good Authority to Occur in Sub-arctic  
Canada, but not Examined by the Author*

*Planorbis (Helisoma) whiteavesi* (Baker (5) ), Lac des Mille Lacs, Thunder Bay district, Ont.

*Planorbis (Helisoma) plexata* Ingersoll (Baker (8) ), Harricanaw River; Black Bear Island, Lake Winnipeg; Cormorant Lake, Man.; Athelstane Lake, Ont.

*Planorbis (Helisoma) hornii* Tryon (Baker (8) ), Fort Simpson, Mackenzie River.

*Planorbis (Helisoma) subcrenatus* Carpenter (Baker (8) ), New Osgoode, Sask.; Wainwright Park, Alta.; Fawn Lake, mouth of Hay River; Mackenzie River, 30 miles above Fort Providence; little lake west end of Great Slave Lake, Mackenzie River district.

*Planorbis (Helisoma) trivolvis macrostomus* Whiteaves (Baker (8) ), several localities in northwestern Ontario; lake northwest of Cormorant Lake, Man.

*Planorbis (Helisoma) campanulatus canadensis* (Baker and Cahn (9) ), Bamaji Lake, Sioux Lookout district, Ont.

*Planorbis (Gyraulus) latistomus* (Baker (5) ), McAree Lake, Rainy River district, Ont.

*Planorbis (Gyraulus) circumstriatus* Tryon (Baker and Cahn (9) ), Winnipeg River, Ont.

*Planorbis (Gyraulus) deflectus obliquus* DeKay (Baker and Cahn (9) ), several localities in Sioux Lookout district, Ont.

#### FAMILY PHYSIDAE

##### Genus *Physa* Draparnaud 1801

##### *Physa gyrina* Say

*Physa gyrina* Say, J. Phil. Acad. 2 : 171. 1821.

*Geographical Range.* North America, Alabama and Texas north to Great Slave Lake.

Northernmost record: Great Slave Lake, N. Lat. 62°, Dall (13).

Southernmost record: Texas.

*Local Distribution.* Previous records: Minaki, Ont., Winnipeg River; Sand and Onion Lakes near Minaki; English River 40 miles north of Minaki; Fox Lake, near Wade; Otter Lake near Minaki, Mozley (21). Ninette, Man., Pelican Lake, Mozley (23). Brereton and Mud Turtle Lakes, Mozley (22). Rainy River, Ont., 1 mile below the Canadian National Railway bridge; Grande Pointe; Onah, Douglas Lake, Man.; Margo, Sask., unnamed lake 1 mile northwest of Whitesand Lake; Kuroki, Fishing Lake; Wadena, Foam Lake; Paskwegin, small lake 2 miles south of Paskwegin siding; Kelliher;

Yonker, Eyehill Creek, Mozley (25). Numerous localities in Jasper National Park, Mozley (26). Sioux Lookout district, Ont., Bamaji, Botsford, Hamilton, Pashkokogan, Cat, Fitchie, Kimmewin, and St. Joseph Lakes, Baker and Cahn (9). Scotsguard, Sask.; Blooming; Beaubier, Russell (36). Tofield, Alta., Beaverhills Lake; Deville, Cooking Lake; Peace River district, Kinuso, Strawberry Creek near Lesser Slave Lake; pond at Mile 200.5, E.D. & B.C. Ry.; Faust, Lesser Slave Lake, Mozley (30). Freshwater and Saltwater Lakes on Charlton Island, James Bay, Richards (35).

*Physa gyrina* is particularly common on the prairie and parkland, and also occurs in the Rocky Mountain region. In the forested territory it is replaced to a great extent by *P. ancillaria*.

*Habitat.* Lakes and ponds.

*Physa gyrina hildrethiana* Lea

*Physa hildrethiana* Lea, Proc. Am. Phil. Soc. 2 : 32. 1841.

*Geographical Range.* North America, Alabama, Pennsylvania, Illinois, north to Canada.

Northernmost record: Winnipeg, Man. Great Slave Lake (Dall) ?

Southernmost record: Alabama.

*Local Distribution.* Winnipeg, Catfish Creek, Mozley (23). Pond in Moose Mountain Forest Reserve, Sask.; Phillips, Alta.; Viking, Mozley (30).

*Habitat.* Temporary ponds and intermittent streams.

*Physa ancillaria* Say

*Physa ancillaria* Say, J. Phil. Acad. 5 : 124. 1825.

*Geographical Range.* North America, New Jersey and Maine, west to Minnesota and Manitoba.

Northernmost record: Madge Lake, north of Kamsack, Sask., N. Lat. 51° 30'.

Southernmost record: "The Ohio River", Baker (3).

*Local Distribution.* New record: stream entering Lake Nipigon from the east, near Macdiarmid, Ont.

Previous record: Madge Lake.

*Habitat.* In the larger lakes, and also sometimes in streams. A common habitat is on smooth rock faces exposed to considerable wave action. The specimens from near Macdiarmid were collected just below a small rapid in the stream.

*Physa integra* Haldeman

*Physa integra* Haldeman, Mon. No. 3, p. 3. 1841.

*Geographical Range.* North America, Ohio and South Dakota, north to Manitoba.

Northernmost record: Victoria Beach, Man., N. Lat. 50° 40'.

Southernmost record: Ohio.

*Local Distribution.* Previous records: Lost and Sword Lakes near Minaki, Ont.; Star Lake near Redditt; Mile 77, G.W.W.D. Ry., Birch River, Man., Mozley (21). Clandeboye, Man., Muckle Creek; Grand Beach, the Grand

Marais; Victoria Beach; Birtle; Birdtail Creek, Mozley (23). Postglacial fossil deposit near Winnipeg, Mozley (31).

*Habitat.* Lakes and streams.

Genus *Aplexa* Fleming. 1820

*Aplexa hypnorum* Linné

*Bulla hypnorum* Linné, Syst. Nat., ed. X, p. 727. 1758.

*Geographical Range.* Europe, northern Asia, North America.

Northernmost record: (in North America) N. Lat. 69° 40', W. Long. 141°.

Southernmost record: "South to the vicinity of the Ohio River", Baker (3).

*Local Distribution.* New records: pond on the Arctic coast of the Yukon Territory at N. Lat. 69° 40', W. Long. 141°, J. M. Jessup coll.; Bilby, Alta.; Vermilion Lake, Banff, Alta.

Previous records: Camden Bay, Arctic coast; also Demarcation Point; pond on Herschel Island; pond at Collinson Point; brackish pond near Teller; Grantley Harbour; Port Clarence, Alaska; pond near Colville mountains, Victoria Island; lake opposite Bernard Harbour; water hole on tundra at Cape Bathurst, Dall (14). Brereton, Man., Mozley (22). Whiteshell River district, Man., above Jessica Lake; Mallard Lake; Little Whiteshell Lake; *Zizania* marsh below the Whiteshell Lakes; near the Seven Sisters Falls, Winnipeg River; Indian Bay station; Molson; Stony Mountain; Victoria Beach; Treesbank; Steep Rock; Sifton; Yonker, Sask., Eyehill Creek, Mozley (24). Rainy River, Ont.; Grande Pointe, Man.; near Clearwater Lake, Hudson Bay Railway; Birtle; Margo, Sask.; Kuroki, Van Patten Creek, Kuroki Creek; Clair, Clair Creek; Watson, Ironspring Creek; Lanigan; Kelliher; Touchwood, near Hudson Bay Lake, Mozley (25). Lake near Jasper, Alta., Mozley (26). Ponds in Moose Mountain Forest Reserve, Sask.; Lindbrook, Alta.; Deville, Cooking Lake; Peace River district, pond at Mile 200.5, E.D. & B.C. Ry.; creek near Faust; 3 miles north of Spirit River. Postglacial fossil deposits near Winnipeg, and on the shore of the Pasquaia River near The Pas, Man., Mozley (31).

*Habitat.* Usually in temporary ponds. Occasionally found in lakes and small slow-flowing streams.

*Physidae Reported on Good Authority to Occur in Sub-arctic  
Canada, but not Examined by the Author*

*Physa jennessi* Dall (14). Bernard Harbour, Arctic Canada.

*P. johnsoni* Clench (12). Hot Sulphur Springs, Banff, Alta.

FAMILY ANCYLIDAE

Genus *Ancylus* Muller 1774

*Ancylus coloradensis* Henderson

*Ancylus coloradensis* Henderson, Nautilus, 44 : 31. 1930.

*Geographical Range.* North America, known from one locality in Colorado, and two in Alberta. Apparently confined to the western mountainous region.

Northernmost record: Two small lakes in the Miette Valley, Jasper National Park, Alta., N. Lat. 53°, Mozley (26).

Southernmost record: Eldora Lake, Boulder Co., Colorado.

*Local Distribution.* The above-mentioned three localities are the only ones known.

*Remarks.* The occurrence of a member of the genus *Ancylus* in North America is of interest since the group in its modern restricted usage is otherwise confined to Europe and northern Asia.

### Genus *Ferrissia* Walker 1903

#### *Ferrissia parallela* (Haldeman)

*Ancylus parallelus* Haldeman, Mon. Pt. 2, p. 3. 1841.

*Geographical Range.* North America, northern Ohio, Nova Scotia, Ontario, and Manitoba.

Northernmost record: Lake Brereton, Man., N. Lat. 50°.

Southernmost record: northern Ohio. "A species of northern distribution", Baker (3).

*Local Distribution.* Previous records: Rennie River, near Brereton, Mozley (22). Whiteshell River, Mozley (24). Pashkokogan Lake, Baker and Cahn (9).

#### *Ferrissia rivularis* (Say)

*Ancylus rivularis* Say, J. Phil. Acad. 1 : 125. 1819.

*Geographical Range.* North America, Ohio and Nebraska north to Canada.

Northernmost record: Birtle, Man., N. Lat. 51° 25'.

Southernmost record: Ohio.

*Local Distribution.* Previous records: Birdtail Creek, Birtle, Mozley. Post-glacial fossil deposit near Winnipeg, Mozley (31).

### *Ancylidae Reported on Good Authority to Occur in Sub-arctic Canada, but not Examined by the Author*

*Ferrissia fusca* (C. B. Adams). Bamaji Lake, Sioux Lookout district, Ont., Baker and Cahn (9).

### FAMILY VIVIPARIDAE

#### Genus *Campeloma* Rafinesque 1819

#### *Campeloma decisum* (Say)

*Limnaea decisum* Say, Nich. Encyc. I, 1817.

*Geographical Range.* North America, New England to Manitoba.

Northernmost record: Berens River, above the junction with the Etomami River, N. Lat. 52° 20'.

Southernmost record: Tennessee.

*Local Distribution.* Previous records: Whiteshell River district, Man., Whiteshell River near first portage below White Lake; between fifth and sixth rapids below Betula Lake; Berens River several miles above the junction

with the Etomami River, Mozley (24). Fort Frances, Ont., Rainy River below the town, also near the town of Rainy River; Grand Beach, Man., the Grand Marais, Mozley (25).

*Viviparidae Reported on Good Authority to Occur in Sub-arctic  
Canada, but not Examined by the Author*

*Campeloma milesii* (Lea). Hamilton Lake, Sioux Lookout district, Ont., Baker and Cahn (9).

FAMILY VALVATIDAE

Genus *Valvata* Muller 1774

*Valvata tricarinata* (Say)

*Cyclostoma tricarinata* Say, J. Phil. Acad. 1 : 13. 1817.

*Geographical Range.* North America, from Virginia and the Ohio River to Alaska.

Northernmost record: Fort Yukon, N. Lat. 66° 40'.

Southernmost record: Virginia.

*Local Distribution.* Previous records: Mackenzie River district, south shore Second Lake, Horn River; southwest side of Mackenzie River 30 miles above Fort Providence; western end of Lake Kakisa; south shore of Lake Kakisa near mouth of Kakisa River, Whittaker (39). St. Norbert, Man., La Salle River; Clandeboye, Muckle Creek; Victoria Beach; Treesbank, Souris River; Theodore, Sask., Whitesand River, Mozley (23). Malachi, Ont., Malachi Lake; Whiteshell River district, Man., Betula Lake; between sixth and seventh rapids below Betula Lake; Morris, Morris River; Delta; Ninette, Bone Lake; Clearwater (Atikameg) Lake, Hudson Bay Railway; Yonker, Sask., Manitou Lake (empty shells only), Mozley (24). Madge Lake north of Kamsack, Sask., Mozley (25). Sioux Lookout district, Ont., outlet of Bamaji Lake, Fitchie, Kimmewin, Botsford, and Cat Lakes, Baker and Cahn (9). Deville, Alta., Cooking Lake; Wabamun, Lake Wabamun, Mozley (30). Post-glacial fossil deposit near Winnipeg, and also near Portage la Prairie and Lavenham, Man., Mozley (31). Moose Factory, Ont., Butler and Charles Islands (recent and fossil), Shipsands Island, James Bay, Richards (35).

*Habitat.* Usually found in lakes, especially in shallow water, sometimes in large ponds and marshes.

*Valvata lewisi* Currier

*Valvata lewisi* Currier, Kent Sci. Inst. Misc. Pub., No. 1, p. 9. 1868.

*Geographical Range.* North America, the northern part of the continent from the Atlantic to the Pacific.

Northernmost record: Bernard Harbour, N. Lat. 68°.

Southernmost record: "Southward range not fully known." Baker.

*Local Distribution.* Previous records: Arctic Coast, creek at Bernard Harbour; lake near Point Williams, Victoria Island, Dall (14). South shore Fawn and Second Lakes, Horn River, Mackenzie River district; southwest side Mackenzie River 30 miles above Fort Providence; south shore Great

Slave Lake near Sulphur Point, Whittaker (39). Numerous localities in Jasper National Park, Alta., Mozley (26). Postglacial fossil deposit on the shore of the Pasquaia River near The Pas, Man., Mozley (31).

*Habitat.* Usually in marshes surrounding relatively large bodies of water.

*Valvata lewisi helicoidea* Dall

*Valvata lewisi* var. *helicoidea* Dall, Harriman Alaska Exped. 13 : 123. 1905. *Geographical Range.* North America, said to occur with the typical *lewisi* throughout its range.

Northernmost record: Fort Yukon, Alaska, N. Lat. 66° 49'.

Southernmost record: Oneida Lake, New York.

*Local Distribution.* Previous records: Mackenzie River district, south shore Second Lake, Horn River; western end Lake Kakisa; Mills Lake at mouth of Horn River; south side Mills Lake, Whittaker (39). Lake Winnipeg, C. H. O'Donoghue coll., Baker (3). Kimmewin Lake, Sioux Lookout district, Baker and Cahn (9).

*Valvatidae Reported on Good Authority to Occur in Sub-arctic  
Canada, but not Examined by the Author*

*Valvata tricarinata perconfusa* Walker, Baker and Cahn (9), outlet of Bamaji Lake, Sioux Lookout district, Ont.

FAMILY AMNICOLIDAE

Genus *Amnicola* Gould and Haldeman 1841

*Amnicola limosa* (Say)

*Paludina limosa* Say, J. Phil. Acad. 1 : 125. 1817.

*Geographical Range.* North America, Texas to New England, Manitoba (and northward ?).

Northernmost record: Lake la Loche, N. Lat. 56° 30', Dall (13) = *limosa*?

The northernmost source of shells seen by the writer is near Winnipeg, Man., (postglacial fossils, Mozley (31) ).

Southernmost record: Texas.

*Local Distribution.* Previous records: Winnipeg; Lake Brereton (*limosa*?) Mozley (22).

*Amnicola limosa porata* (Say)

*Paludina porata* Say, J. Phil. Acad. 2 : 174. 1821.

*Geographical Range.* North America, Illinois to Manitoba. Limits of range not known.

*Local Distribution.* Previous records: Lake Winnipeg, Victoria Beach, Man., Mozley (24). Sioux Lookout district, Ont., Pashkokogan, Hamilton, Kapikik, Cat, Botsford and Kimmewin Lakes, Baker and Cahn (9). Upper Tintah or Norcross Beach of Glacial Lake Agassiz near Lavenham, Man., Mozley (31).

*Amnicola walkeri* Pilsbry

*Amnicola walkeri* Pilsbry, Nautilus, 12 : 43. 1898.

*Geographical Range.* North America, from Ottawa, Ont., to Lake Michigan and Manitoba.

Northernmost record: Lake Winnipeg, Man.

Southernmost record: Lake Michigan ?

*Local Distribution.* Previous records: Victoria Beach, Lake Winnipeg, Mozley (24). Kimmewin Lake, Sioux Lookout district, Ont., Baker and Cahn (9). Upper Tintah or Norcross Beach of Glacial Lake Agassiz near Lavenham, Man., Mozley (31).

*Amnicola winkleyi mozleyi* Walker

*Amnicola winkleyi mozleyi* Walker, Nautilus, 39 : 6. 1925.

*Geographical Range.* North America, Manitoba and Ontario.

Northernmost record: Winnipeg, Man.

*Local Distribution.* Previous records: two postglacial deposits near Winnipeg, Mozley (31).

*Remarks.* A shell which closely resembles this variety was collected in the La Salle River at St. Norbert, Man., but as it is a single specimen it appears best not to cite this as a definite locality until further collections are made.

*Amnicola (Cincinnati) emarginata* (Küster)

*Paludina emarginata* Küster, Paludina, Conch. Cab. p. 50. 1852.

*Geographical Range.* North America, Kentucky and Arkansas north to Canada.

Northernmost record: "N. Lat. 51° on Hudson Bay", Dall (13).

The northernmost source of shells examined by the writer is Winnipeg, Man.

Southernmost record: Arkansas.

*Local Distribution.* Previous records: Mackenzie River district, southwest side of Mackenzie River 30 miles above Fort Providence; south shore Lake Kakisa, near mouth of the Kakisa River; south shore Great Slave Lake near Sulphur Point; south side Mills Lake, Whittaker (39). Postglacial fossil deposit near Winnipeg, Mozley (31).

*Amnicolidae Reported on Good Authority to Occur in Sub-arctic  
Canada but not Examined by the Author*

*Amnicola lustrica decepta* Baker, Botsford Lake, Sioux Lookout district, Ont., Baker and Cahn (9).

*Amnicola (Cincinnati) cincinnatiensis judayi* Baker, Hamilton Lake, Sioux Lookout district, Ont., Baker and Cahn (9).

*Amnicola (Cincinnati) emarginata canadensis* (Baker), Beaver Lake and Little Lake, Mackenzie River district, Baker (3).

**Class PELECYPODA****FAMILY SPHAERIDAE**

All the species of this family which are listed below were identified by the late Dr. Victor Sterki. The records of Baker and Cahn (9) are included, but not those of other earlier authors. It should be noted that Baker and Cahn's material was also examined by Sterki. As there is little reliable information in the literature regarding the range of the members of this family, no geographical notes are given.

*Genus Sphaerium* Scopoli 1777*Sphaerium sulcatum* (Lamarck)

*Cyclas sulcata* Lamarck, An. sans Vert. 5 : 560. 1818.

*Local Distribution.* Previous records: Indian Bay station, Man., Falcon Bay; Rainy Lake, mile east of Wade, Ont., Mozley (21). Theodore, Sask., Whitesand River, Mozley (23). Sioux Lookout district, Ont., Botsford Lake, Baker and Cahn (9). Postglacial deposits near Lavenham, Man., Mozley (31).

*Sphaerium crassum* Sterki

*Sphaerium crassum* Sterki, Nautilus, 14 : 140. 1910.

*Local Distribution.* Backwater of the Winnipeg River, near Minaki, Ont.; Falcon Bay, Shoal Lake, Man., Mozley (21).

*Sphaerium solidulum* (Prime)

*Cyclas solidula* Prime, Proc. Boston Soc. Nat. Hist. 4 : 158. 1851.

*Local Distribution.* Previous records: Mackenzie River district, western end of Lake Kakisa, Whittaker (39). Whitemouth River, Man., near its junction with the Winnipeg River, Mozley (21). Postglacial fossil deposit near Winnipeg, Mozley (31).

*Sphaerium stamineum* (Conrad)

*Cyclas staminea* Conrad, Am. J. Sci. 25 : 342. 1834.

*Local Distribution.* Postglacial deposit near Winnipeg, Man., Mozley (31).

*Sphaerium emarginatum* (Prime)

*Cyclas emarginata* Prime, Proc. Boston Soc. Nat. Hist. 4 : 156. 1851.

*Local Distribution.* Hamilton Lake, Sioux Lookout district, Ont., Baker and Cahn (9); postglacial deposit near Winnipeg, Man., Mozley (31).

*Sphaerium torsum* Sterki

*Sphaerium torsum* Sterki, Annals Carnegie Mus. 10 : 434. 1916.

*Local Distribution.* Postglacial deposit near Winnipeg, Man., Mozley (31).

*Sphaerium acuminatum* (Prime)

*Cyclas acuminata* Prime, Proc. Boston Soc. Nat. Hist. 4 : 155. 1851.

*Local Distribution.* Victoria Beach, Lake Winnipeg, Man., Mozley. Postglacial deposit near Winnipeg, Man., Mozley (31).

*Sphaerium vermontanum* Prime

*Sphaerium vermontanum* Prime, Proc. Acad. Nat. Sci. Phila. p. 128. 1861.

*Local Distribution.* Previous records: Mackenzie River district, western end of Lake Kakisa, Whittaker (39). Sioux Lookout district, Ont., Botsford Lake, Baker and Cahn (9).

*Sphaerium striatinum* (Lamarck)

*Cyclas striatina* Lamarck, An. sans Vert. 5 : 560. 1818.

*Local Distribution.* Previous records: Mackenzie River district, western end of Lake Kakisa, Whittaker (39). Whitemouth River, Man., near its junction with the Winnipeg River, Mozley (21). Postglacial fossil deposit near Winnipeg, Mozley (31).

*Sphaerium occidentale* Prime

*Sphaerium occidentale* Prime, Proc. Acad. Nat. Sci. Phila. p. 295. 1860.

*Local Distribution.* Ditch near Mile 69, G.W.W.D. Ry., Man., Mozley (21).

*Sphaerium tenue* (Prime)

*Cyclas tenuis* Prime, Proc. Boston Soc. Nat. Hist. 4: 161. 1851.

*Local Distribution.* Previous records: Mackenzie River district, south shore Second Lake, Fawn River; southwest side of Mackenzie River 30 miles above Fort Providence; south shore of Lake Kakisa near mouth of the Kakisa River; south side of Mills Lake, Whittaker (39). Traverse Bay, near Victoria Beach, Lake Winnipeg, Man., Mozley (23). Sioux Lookout district, Ont., Bamaji Lake, Baker and Cahn (9).

*Sphaerium notatum* Sterki

*Sphaerium notatum* Sterki, Nautilus, 41 : 55. 1927.

*Local Distribution.* Postglacial deposit near Winnipeg, Man., Mozley (31).

Genus *Musculium* Link 1907*Musculium transversum* (Say)

*Cyclas transversa* Say, New Harmony Diss. 2 : 356. 1829.

*Local Distribution.* Previous records: Matlock, Lake Winnipeg, Man., Mozley (23). Postglacial deposit near Winnipeg, Mozley (31).

*Musculium truncatum* (Linsley)

*Cyclas truncata* Linsley, Am. J. Sci. 6 : 234. 1848.

*Local Distribution:* Previous records: Hamilton Lake, Sioux Lookout district, Ont.; Skunk Lake, near Minaki, Ont., Mozley (21); marsh bordering Lake Winnipeg, near Victoria Beach, Man., Mozley (23).

*Musculium rosaceum* (Prime)

*Cyclas rosacea* Prime, Proc. Boston Soc. Nat. Hist. 4 : 155. 1851.

*Local Distribution.* Previous records: Mackenzie River district, south side Mills Lake, Whittaker (39). Sioux Lookout district, Ont., St. Joseph,

Botsford, Hamilton, Kimmewin, Kapikik and Pashkokogan Lakes, Baker and Cahn (9).

*Musculium ryckholti* (Normand)

*Cyclas ryckholti* Normand, Notes sur Quelques Nouvelles Cyclades, p. 7. 1844.

*Local Distribution.* Previous records: marsh on shore of Lake Winnipeg at Victoria Beach, Man.; small lake 1 mile northeast of Ninette Sanitorium, Ninette, Man., Mozley.

*Musculium securis* (Prime)

*Cyclas securis* Prime, Proc. Boston Soc. Nat. Hist. 4 : 160. 1851.

*Local Distribution.* Previous records: Alice Lake, between Minaki and Wade, Ont., Mozley (21). Lake Brereton, Man., Mozley (22). Sioux Lookout district, Ont., Fitchie Lake, Baker and Cahn (9).

Genus *Pisidium* C. Pfeiffer 1821

*Pisidium compressum* Prime

*Pisidium compressum* Prime, Proc. Boston Soc. Nat. Hist. 4 : 161. 1851.

*Local Distribution.* South side Mills Lake, Mackenzie River district, Whitaker (39). Birdtail Creek near Birtle, Man.; Whitesand River near Theodore, Sask., Mozley (23). Lake Annette near Jasper, Alta.; Lake No. 2 near Geikie, Alta., Mozley (26). Postglacial deposit near Winnipeg, Man., Mozley (31). *P. compressum* var. Pashkokogan and Botsford Lakes, Sioux Lookout district, Ont., Baker and Cahn (9).

*Pisidium fallax* Sterki

*Pisidium fallax* Sterki, Nautilus, 10 : 20. 1896.

*Local Distribution.* Whitesand River near Theodore, Sask., Mozley (23). Postglacial deposit near Winnipeg, Man., Mozley (31).

*Pisidium punctatum* Sterki

*Pisidium punctatum* Sterki, Nautilus, 7 : 99. 1895.

*Local Distribution.* Hamilton Lake, Sioux Lookout district, Ont., Baker and Cahn (9).

*Pisidium variabile* Prime

*Pisidium variabile* Prime, Proc. Boston Soc. Nat. Hist. 4 : 163. 1851.

*Local Distribution.* Lake No. 5 near Geikie, Alta., Mozley (26). Botsford Lake, Sioux Lookout district, Ont., Baker and Cahn (9).

*Pisidium variabile brevius* Sterki

*Pisidium variabile brevius* Sterki, Nautilus, 19 : 118. 1906.

*Local Distribution.* Hibernia Lake and Caledonia Creek, near Jasper, Alta., Mozley (26).

*Pisidium minusculum* Sterki

*Pisidium minusculum* Sterki, Nautilus, 20 : 17. 1906.

*Local Distribution.* Kimmewin Lake, Sioux Lookout district, Ont., Baker and Cahn (9).

*Pisidium adamsi* Prime

*Pisidium adamsi* Prime, Smithsonian Misc. Coll. 145 : 16. 1865.

*Local Distribution.* Lake Brereton, Man., Mozley (22). Cat and Fitchie Lakes, Sioux Lookout district, Ont., Baker and Cahn (9).

*Pisidium neglectum* Sterki

*Pisidium neglectum* Sterki, Nautilus, 20 : 87. 1906.

*Local Distribution.* Jacques Lake, and the stream which drains it (not Jacques Creek), Rocky River drainage, northeast of Jasper, Alta., Mozley (26).

*Pisidium scutellatum* Sterki

*Pisidium scutellatum* Sterki, Nautilus, 10 : 66. 1896.

*Local Distribution.* Mackenzie River district, south shore of Fawn and Second Lakes, Horn River; southwest side of Mackenzie River 30 miles above Fort Providence; south shore Lake Kakisa, near mouth of the Kakisa River; south side Mills Lake, Whittaker (39). Lakes Annette and Patricia, near Jasper, Alta., Mozley (26). Bamaji, Hamilton, Pashkokogan and Kimmewin Lakes, Sioux Lookout district, Ont., Baker and Cahn (9).

*Pisidium roperi* Sterki

*Pisidium roperi* Sterki, Nautilus, 12 : 77. 1898.

*Local Distribution.* Pond near Mud Turtle Lake, Man., Mozley (22). Marsh on shore of Lake Winnipeg, near Victoria Beach, Mozley (23). Lake No. 8, near Jasper, Alta., Mozley (26).

*Pisidium subrotundum* Sterki

*Pisidium subrotundum* Sterki, Nautilus, 20 : 19. 1906.

*Local Distribution.* Small lake near Ninette, Man., Mozley (23).

*Pisidium splendidulum* Sterki

*Pisidium splendidulum* Sterki, Nautilus, 11 : 113. 1898.

*Local Distribution.* Lake No. 10, near Jasper, Alta., Mozley (26).

*Pisidium tenuissimum* Sterki

*Pisidium tenuissimum* Sterki, Nautilus, 14 : 99. 1901.

*Local Distribution.* Skunk Lake, near Minaki, Ont., Mozley (21). Lake Edith and the larger Trefoil Lake, near Jasper, Alta., Mozley (26).

*Pisidium rotundatum* Prime

*Pisidium rotundatum* Prime, Proc. Boston Soc. Nat. Hist. 4 : 164. 1851.

*Local Distribution.* Hamilton and Kimmewin Lakes, Sioux Lookout district, Ont., Baker and Cahn (9).

*Pisidium ferrugineum* Prime

*Pisidium ferrugineum* Prime, Proc. Boston Soc. Nat. Hist. 4 : 162. 1851.

*Local Distribution.* Hamilton Lake, Sioux Lookout district, Qnt., Baker and Cahn (9).

*Sphaeriidae Reported on Good Authority to Occur in Sub-arctic  
Canada, but not Examined by the Author (Identifications by Sterki)*

*Sphaerium tumidum* Baird, south shore Great Slave Lake, near Sulphur Point (?); near Fort Wrigley, Mackenzie River district, Whittaker (39).

*Musculium jayense* Prime, south shore Fawn Lake, Horn River, Mackenzie River district, Whittaker (39).

*Pisidium indianense* Sterki, south shore Second Lake, Horn River; at mouth of the Hay River, Mackenzie River district, Whittaker (39).

FAMILY UNIONIDAE

Genus *Quadrula* Rafinesque 1820

*Quadrula quadrula* Rafinesque

*Obliquaria (Quadrula) quadrula* Rafinesque, Ann. Sci. Phys. Bruxelles, 5 : 307-1820.

*Geographical Range.* North America, the Mississippi and St. Lawrence drainages; Canada east of the Rocky Mountains.

Northernmost record: Red River at Winnipeg.

Southernmost record: eastern Texas.

*Local Distribution.* Red River at Winnipeg.

Genus *Amblema* Rafinesque 1819

*Amblema costata* (Rafinesque)

*Amblema costata* Rafinesque, Ann. Sci. Phys. Bruxelles, 5 : 315. 1820.

*Geographical Range.* North America, the Mississippi and St. Lawrence drainages; Canada east of the Rocky Mountains.

Northernmost record: Saskatchewan River, Lake Winnipeg.

Southernmost record: Alabama River ? Southern distribution imperfectly known.

*Local Distribution.* New record: near the Englishman's Rapid, Berens River, Man., at a depth of 12 metres.

Previous records: Red, and Black Rivers, Man.

Genus *Fusconaia* Simpson 1900

*Fusconaia flava* (Rafinesque)

*Obliquaria flava* Rafinesque, Ann. Sci. Phys. Bruxelles, 5 : 305. 1820.

*Geographical Range.* North America, the Mississippi drainage; Canada east of the Rocky Mountains.

Northernmost record: Nelson River, Man.

Southernmost record: West Virginia ?

*Local Distribution.* Red, Roseau, Souris, and Nelson Rivers; Lake Winnipeg.

Genus *Strophitus* Rafinesque 1820

*Strophitus rugosus* (Swainson)

*Anodon rugosus* Swainson, Zool. Ill., Ser. I, II, pl. 96. 1822.

*Geographical Range.* North America, the Mississippi drainage; Canada east of the Rocky Mountains.

Northernmost record: mouth of the Hay River, N. Lat. 60° 51'.

Southernmost record: Arkansas.

*Local Distribution.* New record: Birdtail Creek, near Birtle, Man.

Previous records: Red, Whitemouth and Saskatchewan Rivers; Lake Winnipeg; Playgreen Lake.

Fossil records: Lake Agassiz clay near Mile 93, G.W.W.D.Ry., Man.; post-glacial gravel bed near Birtle, Man.

*Remarks.* Baker (3) states that specimens from Arkansas and elsewhere belong to the variety *pavonius* of Lea.

#### Genus *Anodonta* Lamarck 1799

##### *Anodonta grandis* Say

*Anodonta grandis* Say, New Harmony Diss. 2 : 341. 1829.

*Geographical Range.* North America, Mississippi drainage and upper St. Lawrence drainage; Canada east of the Rocky Mountains.

Northernmost record: Lake Winnipeg ?

Southernmost record: Texas.

*Local Distribution.* New record: Morris River, near Morris, Man. Previously reported from the Fairford River and other localities, most of the records probably being based upon the sub-species mentioned below.

##### *Anodonta grandis footiana* Lea

*Anodonta footiana* Lea, Proc. Am. Phil. Soc. 1 : 289. 1840.

*Geographical Range.* North America, Mississippi drainage; Canada east of the Rocky Mountains.

Northernmost record: Lake Kakisa, N.W.T., N. Lat. 61°.

Southernmost record: Michigan ?

*Local Distribution.* New record: Birdtail Creek near Birtle, Man.

Previous records: numerous lakes in far western Ontario; Red and Souris Rivers, Man.; Battle River Lake, Alta.

Fossil record: postglacial gravel bed near Birtle.

*Remarks.* The records of *A. grandis* from Lake Winnipeg may refer to this variety.

##### *Anodonta kennicotti* Lea

*Anodonta kennicotti* Lea, Proc. Phil. Acad. 5 : 56. 1861.

*Geographical Range.* North America, St. Lawrence, Hudson Bay, and Mackenzie drainages.

Northernmost record: outlet of Lake Winnipeg.

Southernmost record: Lake of the Woods ?

*Local Distribution.* New records: Lake Brereton, Man. (previously reported as *A. grandis footiana*); Lake Winnipegosis.

Previous records: numerous lakes and streams in far western Ontario; Lakes Winnipeg and Manitoba; Grand Rapids of the Saskatchewan; Ekwan River, Keewatin.

Genus *Anodontoides* Simpson 1898*Anodontoides ferussacianus* (Lea)

*Anodonta ferussaciana* Lea, Trans. Am. Phil. Soc. 5 : 45. 1834.

*Geographical Range.* North America, Mississippi and St. Lawrence drainages; Canada east of the Rocky Mountains.

Northernmost record: Whitesand River, near Theodore, Sask.

Southernmost record: Tennessee.

*Local Distribution.* New record: Whitesand River, as above.

Previous records: Lake of the Woods; Lake Winnipeg.

Genus *Lasmigona* Rafinesque 1831*Lasmigona complanata katherinae* (Lea)

*Unio katherinae* Lea, Syn., p. 35. 1838.

*Geographical Range.* North America, the northern part of the Mississippi drainage?, Lake Superior, and Canada east of the Rocky Mountains; Hudson Bay drainage.

Northernmost record: Lake Winnipeg; "Nelson River drainage", Dall.

Southernmost record: Lake Superior; northern part of Mississippi drainage?

*Local Distribution.* New records: Birdtail Creek near Birtle, Man.; Moose Jaw Creek near Moose Jaw, Sask.

Previous records: Winnipeg River at Minaki; Lake of the Woods, Ont.; Roseau, Souris, Red, Assiniboine, and Nelson Rivers, Man.; Lake Winnipeg; lower Saskatchewan River, Shoal, Shell, and Battle Rivers, Battle River Lake, Alta.; Carrot River near Arborfield, Sask.

Previous fossil record: postglacial gravel bed near Birtle.

*Lasmigona compressa* (Lea)

*Symphynota compressa* Lea, Trans. Am. Phil. Soc. 3 : 450. 1829.

*Geographical Range.* North America, New York to Nebraska, and from Kentucky to Manitoba and Saskatchewan.

Northernmost record: Carrot River near Arborfield, Sask.

Southernmost record: Kentucky.

*Local Distribution.* New fossil record: postglacial gravel bed near Birtle, Man.

Previous records: Boulder and Missinaibi Rivers, Ont.; Carrot River.

*Remarks.* This species has been reported from the Shenyenne River south of Devils Lake, North Dakota, by Winslow (40). This stream is in the drainage basin of the Red River.

Genus *Proptera* Rafinesque 1819*Proptera alata megaptera* (Rafinesque)

*Metaptera megaptera* Rafinesque, Ann. Sci. Phys. Bruxelles, 5 : 307. 1820.

*Geographical Range.* North America, Mississippi and (in part) Atlantic drainages; Canada east of the Rocky Mountains.

Northernmost record: Red River, Man.

Southernmost record: northern Alabama.

*Local Distribution.* Previous records: near Lake of the Woods, Ont.; Red River.

*Remarks.* Following Baker (3), the above name is used for the species formerly known as *Lampsilis alatus* (Say).

Genus *Actinonaias* Fischer and Crosse 1893

*Actinonaias carinata* (Barnes)

*Unio carinatus* Barnes, Am. J. Sci. 6 : 259. 1823.

*Geographical Range.* North America, Mississippi drainage; Canada east of the Rocky Mountains; for details of range in the vicinity of the Great Lakes see Baker (3, p. 221).

Northernmost record: Assiniboine River, Man.

Southernmost record: southern Michigan; Arkansas ?

*Local Distribution.* New record: Assiniboine River at Treesbank, Man.

Previous records: Roseau River; Assiniboine River at Millwood, Man.

Genus *Ligumia* Swainson 1840

*Ligumia recta latissima* (Rafinesque)

*Unio latissima* Rafinesque, Ann. Sci. Phys. Bruxelles, 5 : 297. 1820.

*Geographical Range.* North America, Mississippi and Atlantic drainages; western Canada east of the Rocky Mountains.

Northernmost record: Red River, Man.

Southernmost record: Georgia and Arkansas.

*Local Distribution.* New record: Assiniboine River at Aweme, Man.

Previous records: Lake of the Woods, Ont.; Roseau, Red, and Assiniboine Rivers, Man.

Fossil record: postglacial gravel bed near Birtle, Man.

Genus *Lampsilis* Rafinesque 1820

*Lampsilis ventricosa* (Barnes)

*Unio ventricosus* Barnes, Am. J. Sci. 6 : 267. 1823.

*Geographical Range.* North America, Mississippi and Atlantic drainages; Canada east of the Rocky Mountains.

Northernmost record: Nelson River, Man.

Southernmost record: Oklahoma.

*Local Distribution.* New record: Nelson River, at the point where the Hudson Bay Railway crosses that stream for the first time north of The Pas, Man.

Previous records: Roseau and Red Rivers; Lake Winnipeg.

Previous fossil record: postglacial gravel bed near Birtle, Man.

*Remarks.* *L. ventricosa* is represented in this part of North America by a dwarf variety, but no useful purpose would be served, at least for the present, by describing it as a new sub-species.

Baker (3) regards the great majority of the previous records of this species in Wisconsin as belonging to the variety *occidens* (Lea). Having examined the type specimen of this variety in the collection of the United States National Museum in conjunction with his own and other specimens, the author is not convinced that it is a distinct race.

*Lampsilis siliquioidea rosacea* (De Kay)

*Unio rosacea* De Kay, Zool. N.Y. 5 : 192. 1843.

*Geographical Range.* North America, Mississippi and Atlantic drainages; Canada east of the Rocky Mountains.

Northernmost record: Great Slave Lake.

Southernmost record: northern Indiana.

*Local Distribution.* New records: Whiteshell River, several localities below Betula Lake; Birdtail Creek near Birtle; Valley River, 1 mile above its mouth; Mossy River near Lake Winnipegosis; Lake Winnipegosis, all in Manitoba.

Previous records: this species has been reported from many localities throughout western Canada to the east of the Rocky Mountains.

*Remarks.* Gravid specimens were collected in Birdtail Creek on November 11, 1924, and in the Red River near Winnipeg on November 1, 1925.

*Lampsilis superiorenensis* (Marsh)

*Unio superiorenensis* Marsh, Nautilus, 10 : 103. 1897.

*Geographical Range.* North America, from the region of Lake Superior to the Mackenzie River district.

Northernmost record: Lake Kakisa, N. Lat. 61°.

Southernmost record: the upper Great Lakes region.

*Local Distribution.* Previous records: numerous localities in far western Ontario. Lake Kakisa, and the mouth of the Hay River, Mackenzie River district, Whittaker (39).

*Unionidae Reported on Good Authority to Occur in Sub-arctic  
Canada, but not Examined by the Author*

*Anodonta marginata* Say, Schist Lake, Sioux Lookout district, Ont., Baker and Cahn (9). This locality is in the Hudson Bay drainage. It is of interest as possibly indicating a westward migration of this characteristic Atlantic coast species. Baker and Cahn state that the single specimen collected was typical in form and color.

### The Local Distribution of the Molluscan Fauna

The general procedure in this work has been to ascertain the types of habitat which are available in this region for settlement by molluscs, and then to observe the distribution of the species in them. It has been considered desirable to define briefly the position which this part of Canada occupies with reference to the adjoining regions. A brief summary of the various zoogeographical sub-divisions of North America is therefore given.

In most of the previous work on the flora and fauna of North America, attention has been directed chiefly to the details of distribution rather than to general geographical considerations. It may not be out of place to devote a paragraph to showing that the various schemes of classification which have been proposed are not mutually exclusive, as has sometimes been supposed, but are complementary.

#### THE ZOOGEOGRAPHICAL SUB-DIVISIONS OF NORTH AMERICA

It is now generally agreed among biologists and geographers that North America forms a distinct zoogeographical region. Regarding the sub-division of this continent however, there is wide divergence of opinion. These differences appear to have arisen, at least in part, from the diverse points of view from which the subject has been approached. The "Life Zones" of Merriam (19) afford an example, being based upon a conception very similar to that of the "heat budget," *i.e.*, the total quantity of heat required to produce mature individuals of a given organism and to ensure reproduction. Merriam's Life Zones have the disadvantage of being somewhat arbitrary and inflexible. A useful alternative division of the continent is into Arctic, Sub-arctic, and Temperate belts or regions. This takes into account the facts which formed the basis of the division of North America into Holarctic and Sonoran portions by Lydekker (18), and there can be little doubt that there is a change in the character of the fauna of the continent in the neighborhood of N. Lat. 50°. Those who approach the subject from the standpoint of ecology place much importance upon the natural developmental cycles of the vegetation, and the associated changes in the fauna. It is also desirable that the existence of widespread natural regions or prevailing landscape types should be kept in mind. According to this conception, the northern part of the continent at least may be divided into a number of natural areas, in which the climate, soils, flora, and fauna are similar within sufficiently narrow limits to give the country an essential homogeneity and characteristic appearance. Examples of these natural regions are the barren ground, northern coniferous forest, parkland, and prairie. It is likely that there is a degree of truth in each of these viewpoints.

The region dealt with in this account lies in the sub-arctic region, covers four natural regions or major landscape types, and includes four of Merriam's Life Zones. A consideration of some of the habitats of this region from a developmental standpoint is to be found in the work of Bird (10).

#### TYPES OF HABITAT FOUND IN SUB-ARCTIC CANADA AND THEIR MOLLUSCAN FAUNA

Up to the present, most of the published accounts relating to the freshwater mollusca of this region have been in the nature of faunal lists. Little attention has been paid to the local distribution of the species. Several studies of the habitat relationships of the mollusca have been made in the United States, but only one short account has been published of such work,

carried out within a radius of several hundred miles, in western Canada. Most investigations in other parts of the world have been concerned with the distribution of the mollusca in a single body of water, and the geographical aspects of the subject have been neglected. Although much important information has resulted from ecological studies, many of them tend to convey an impression, whether intentionally or not, of the existence of a high degree of uniformity in the fauna of many types of aquatic habitats. This is erroneous. The fauna of such situations is not stereotyped, nor is the association of the various species a haphazard affair. In another paper (Mozley (34) ) it has been demonstrated that this association may be the result of definite, though complex reactions, and can be measured and mathematically expressed. The diversity in the constitution of the fauna of similar habitats is very noticeable throughout the sub-arctic region. Under different geographical conditions it is apparently possible for one species to occupy the position of another in the environmental complex, with considerable precision. This probably involves the substitution of the one species for the other in the food chains. Investigations along these lines promise interesting results.

Within the territory included in the scope of this paper there are four well-marked natural regions, known as the Prairie, Parkland, Forest, and Barren Ground. These are comparable respectively to the Steppe, Forest-Steppe, Taiga, and Tundra of northern Asia.

The Prairie, which is situated along the southern boundary of western Canada, is characterized by the predominance of grassy vegetation and the absence of trees. Many of the large lakes of this region are saline, and the most favorable habitats for fresh-water mollusca are small marshy lakes. Ponds are numerous, but, as a result of the semi-arid climate, many of them contain water only during the spring of each year.

The Parkland, which is found to the north of the Prairie, is a narrow belt of groves of aspen (*Populus tremuloides*) with intervening grassy glades. As far as fresh-water organisms are concerned, conditions on the Parkland are very similar to those on the Prairie, but many of the Parkland waters are less saline. Ponds are somewhat more numerous, and they contain water for a longer period each year than do those on the Prairie. The general conditions of life in these two regions closely resemble those on the Steppe of northern Kazakstan, and the Forest-Steppe of Siberia. For the most part, the species of animals which occupy similar situations in Asia and North America are different, although frequently of the same or related genera. Ecologically they are closely comparable.

The Forest, which forms a broad belt running across the whole of the country from Ungava to the Rocky Mountains, presents conditions which are unlike those of the Prairie and Parkland. The waters of this area are neutral or acid in reaction. In many instances they have a low content of dissolved mineral salts, and are relatively rich in organic matter. *Sphagnum* invades many of the smaller bodies of water in this region and appears to have an unfavorable effect upon the molluscan fauna, or at least to be associated with

such an effect. In this respect many of the small lakes and ponds of this region resemble those of northern Sweden and Finland. It is a notable fact that when such waters are aerated, as in streams with rapids and falls, they appear to be capable of supporting a much richer molluscan fauna. Large rock-bound lakes with clear and cold waters are not uncommon in the forested region, and they have a characteristic molluscan fauna.

The Barren Ground, which lies to the north of the forested area along the Arctic coast of Canada, is a treeless area which closely resembles the Tundra of Fennoscandia and Siberia. Several fresh-water mollusca are found in this region in abundance.

The habitats of fresh-water mollusca in sub-arctic Canada may be grouped under seven descriptive headings, namely:

- (i) Temporary ponds.
- (ii) Ponds which contain water permanently, and small shallow lakes.
- (iii) Large lakes having outlet streams.
- (iv) Lakes without direct outlet streams.
- (v) Intermittent streams.
- (vi) Permanent streams of the non-mountainous region.
- (vii) Streams of the Rocky Mountain region.

This grouping of habitats is somewhat arbitrary, and takes no account of the fact that each pond, lake and stream has its own individual characteristics. There are also numerous habitats which are not easily placed in one of the above classes. Nevertheless it is desirable to have clear-cut conditions as the basis of the classification, even though this tends in some instances to exaggerate the importance of extreme conditions.

The plan of presentation adopted in the following account is to give a brief description of some of the salient features of the principal habitat types, with comments on their fauna in other parts of the sub-arctic region. This is followed by a series of concrete examples in which the molluscan fauna of certain specific localities is listed. It should be borne in mind that these molluscan associations are not necessarily typical, but taken as a whole they are believed to be representative. All the information is original and has been drawn from a large number of observations.

#### TEMPORARY PONDS

The presence of a large number of small, shallow ponds is characteristic of many parts of the Prairie and Parkland. These usually occupy little depressions in which water from the melting snow collects in the spring, and remains through the months of April, May, and in some instances a part of June. After this they are dry until the following spring. Such ponds are characterized by a short aquatic phase in the spring followed by progressively drier conditions, and are subject to low temperatures during the winter. A description of the aquatic fauna and general conditions in one pond of this type has already been published (Mozley, 27). Similar ponds occur on the Steppe and Forest-Steppe of northern Asia. It may be worth noting that such

ponds in Canada and Siberia draw most of their water supply from melting snow, and this may be of significance in comparing similar habitats in South Africa and Australia which are fed by rains.

Apart from certain molluscs, namely *Planorbis umbilicatellus*, and *Planorbula campestris*, the animals which are most characteristic of these situations in Canada are phyllopod crustacea such as *Lepidurus*, *Branchipus*, *Limnetis*, and *Estheria*.

#### Examples

1. A small pond between Lake Brereton and Mud Turtle Lake, eastern Man. *Aplexa hypnorum*.
2. A small pond near the shore of Mud Turtle Lake. *Planorbis umbilicatellus*.
3. A small pond near Stony Mountain, Man. *Lymnaea palustris*, *Aplexa hypnorum*.
4. A pond 4 miles southwest of Winnipeg, Man. *Lymnaea caperata*, *Planorbula campestris*, *Aplexa hypnorum*.
5. A pond near Spirit River, Alta. *Lymnaea palustris*, *L. caperata*, *Planorbis umbilicatellus*, *Aplexa hypnorum*.
6. A pond near St. Vital, Man. *Lymnaea palustris*, *L. caperata*, *Planorbis exacuus*, *P. umbilicatellus*, *Planorbula campestris*, *P. crassilabris*, *Aplexa hypnorum*.

#### SMALL FRESH-WATER LAKES AND PONDS

Under this heading are included ponds which contain water permanently, and small pond-like lakes. In regions where there are few outcrops of the bedrock, such bodies of water generally have low muddy shores, and are invaded to a greater or less extent by marsh plants such as *Scirpus* and *Typha*. Under these conditions the predominant molluscs are *Lymnaea stagnalis jugularis*, *L. palustris*, *Planorbis trivolvis*, and *Physa gyrina*. In parts of the forested regions the lakes have rocky shores, and under these conditions the tendency towards marsh development is less marked. The species found most commonly in these rock-bound lakes are *Planorbis campanulatus wisconsinensis*, *P. corpulentus*, and *Physa ancillaria*. In shallow places where there is decaying vegetable matter *Lymnaea megasoma* is sometimes to be found in abundance. A particular type of pond, formed by beavers (*Castor canadensis*) is of considerable local importance as a habitat for molluscs in the Rocky Mountain area (Mozley (28)).

The small lakes of Canada closely resemble those of Siberia. In the forested part of the latter country, however, a greater proportion of the small basins are filled to such an extent with vegetation that they are no longer suitable habitats for fresh-water mollusca. Ponds on the flood plains of streams are less important habitats for mollusca in Canada than in northern Asia. Among the fresh-water gastropods which are common to these two countries are *Lymnaea stagnalis* and *L. palustris*. The species which in Canada most frequently accompanies *L. stagnalis* in ponds and small lakes is *Planorbis trivolvis*. In Siberia this position is taken by *P. corneus*. *Lymnaea palustris*

is not as common in Siberia as in Canada, and possibly it is displaced to some extent in the pond fauna of the former country by *Lymnaea pereger*, a species which does not occur in North America.

#### Examples

1. A pond at Mile 95, G.W.W.D. Ry., Man. *Lymnaea lanceata*.
2. The smaller Trefoil Lake, near Jasper, Alta. *Planorbis crista*.
3. Lake Dorothy, Miette Valley, Jasper. *Physa gyrina*.
4. Rainy Lake, near Wade, Ont. *Sphaerium sulcatum*.
5. A pond on Duck Mountain, 20 miles east of Kamsack, Sask. *Lymnaea palustris*, *Planorbis trivolvis*.
6. A pond formed by beavers on Tekarra Creek, Jasper. *Planorbis arcticus*, *Physa gyrina*.
7. Skunk Lake, near Minaki, Ont. *Sphaerium truncatum*, *Pisidium tenuissimum*.
8. Sword Lake, near Minaki. *Planorbis campanulatus wisconsinensis*, *Physa integra*.
9. Viri Lake, Miette Valley, Jasper. *Planorbis crista*, *Physa gyrina*.
10. Low-lying ground near Round Lake, Ninette, Man. *Lymnaea humilis modicella*, *L. obrussa exigua*.
11. Alice Lake, near Minaki. *Planorbis campanulatus wisconsinensis*, *P. arcticus*, *Musculium securis*.
12. A pond formed by beavers on a small stream crossing Buffalo Prairie, Athabasca Valley, near Jasper. *Lymnaea stagnalis wasatchensis*, *L. traskii*, *Planorbis arcticus*, *Physa gyrina*.
13. A pond near the shore of Hudson Bay, east of Fort Churchill, Man. *Lymnaea palustris*, *Planorbis arcticus*, *Physa gyrina*, *Aplexa hypnorum*.
14. A pond on Moose Mountain, southern Sask. *Lymnaea stagnalis jugularis*, *L. palustris*, *Planorbis trivolvis*, *P. exacuouus*, *Physa gyrina*.
15. A small lake near Cottonwood Creek, Jasper. *Lymnaea stagnalis wasatchensis*, *L. emarginata canadensis*, *L. dalli*, *Planorbis trivolvis*, *P. hirsutus*, *P. arcticus*, *Pisidium roperi*, *P. splendidulum*.
16. "Lake No. 1", Miette Valley, Jasper. *Planorbis exacuouus*, *P. hirsutus*, *P. arcticus*, *Physa gyrina*, *Ancylus coloradensis*, *Valvata lewisi*, *Pisidium* sp.
17. Lake Mildred, near Jasper. *Lymnaea stagnalis wasatchensis*, *L. palustris*, *L. obrussa decampi*, *Planorbis arcticus*, *Physa gyrina*, *Valvata lewisi*.
18. Douglas Lake, near Onah, Man. *Lymnaea stagnalis jugularis*, *L. palustris*, *Planorbis campanulatus davisii*, *P. trivolvis*, *P. exacuouus*, *Physa gyrina*.
19. The larger Trefoil Lake, Jasper. *Lymnaea stagnalis wasatchensis*, *Planorbis trivolvis*, *P. exacuouus*, *P. arcticus*, *Physa gyrina*, *Pisidium tenuissimum*.
20. Pelican Lake, Ninette, Man. (i) On *Potamogeton* near the centre of the lake, *Physa gyrina* (abundant), *Lymnaea palustris* (less common); (ii) in *Typha* and *Scirpus* marsh around the shore of the lake, *Lymnaea*

*stagnalis jugularis*, *L. palustris*, *Planorbis exacuus*, *P. arcticus*, *Planorbula armigera*, *Physa gyrina*; (iii) in a moist meadow on slightly higher ground near the marsh, *Lymnaea caperata*, *L. parva* var., *Aplexa hypnorum*.

#### LARGE LAKES HAVING OUTLET STREAMS

Large lakes are numerous in sub-arctic Canada, and their outstanding characteristic is that they are of moderate depth (commonly up to 30 m.), and that their shores are subject to severe wave action. In places where the shores are protected either as a result of their configuration or the presence of islands, the molluscan fauna is similar to that found in smaller bodies of water. The most typical species on exposed shores are *Lymnaea stagnalis sanctaemariae*, *L. emarginata*, and *L. catascopium*. Fresh-water mussels are to be found on the bottom of many lakes, the commonest species being *Lampsilis siliquioidea rosacea* and *Anodonta kennicotti*.

Few comparable habitats are to be found in Siberia, where there is little or no adaptation in the pulmonate fauna to meet lacustrine conditions such as is found in Canada. The fauna of Lake Baikal, while lacustrine, is of such a special type that no parallel can be drawn between it and any Canadian lake. *Lymnaea stagnalis sanctaemariae* of Canada is a variety which occupies a habitat very similar to that of *L. stagnalis lacustris* in Europe, and the shells of the two varieties have a certain resemblance to each other. *Lymnaea stagnalis lillianae* may also have a parallel form in Europe, e.g., the large variety of *L. stagnalis* which inhabits Lohja Lake in southern Finland.

#### Examples

1. Maligne Lake, 30 miles east of Jasper, Alta. Altitude 5555 ft. *Lymnaea traskii*.
2. Pyramid Lake, near Jasper. Altitude 3867 ft. *Lymnaea stagnalis wasatchensis*, *Planorbis antrosus sayi*, *P. exacuus*, *P. hirsutus*, *P. arcticus*, *Physa gyrina*, *Pisidium* spp.
3. Atikameg Lake, Mile 17, Hudson Bay Railway, Man. *Lymnaea emarginata* var., *L. obrussa decampi*, *Planorbis exacuus*, *P. deflectus*, *P. hirsutus*, *Valvata tricarinata*.
4. Shoal Lake, eastern Man. (i) Exposed rocky shores of Indian Bay, *Physa ancillaria*; (ii) sandy shore of Indian Bay somewhat exposed to wave action, *Lymnaea stagnalis lillianae*; (iii) protected shore of Indian Bay, *Lymnaea obrussa exigua*; (iv) Falcon Bay in quiet water, on sand bottom and in small marshes, *Lymnaea stagnalis jugularis*, *Planorbis campanulatus wisconsinensis*, *P. trivolvis pilsbryi*, *P. antrosus sayi*, *P. exacuus*, *P. hirsutus*, *P. arcticus*, *Planorbula crassilabris*, *Sphaerium crassum*, *Anodonta kennicotti*.
5. Lake Brereton, Man. (i) On exposed rocky shores subject to severe wave action, *Lymnaea stagnalis sanctaemariae*, *Planorbis antrosus sayi*, *P. campanulatus wisconsinensis*, *Physa gyrina*; (ii) on partly protected rocky shores, *Lymnaea columella casta*, *Planorbis exacuus*; (iii) on

partly protected sandy shores, chiefly on *Potamogeton*, *Amnicola limosa*; (iv) on *Potamogeton* and other plants in bays protected from wave action, *Lymnaea megasoma*, *Planorbula crassilabris*, *Pisidium adamsi*; (v) in *Typha* marsh at the mouth of the Rennie River, *Lymnaea megasoma*, *L. lanceata*, *Planorbis antrosus sayi*, *P. exacuus*, *P. arcticus*, *P. hirsutus*, *Physa gyrina*, *Ferrissia parallela*, *Musculium securis*.

6. Lake Winnipeg, near Victoria Beach, Man. (i) On shore stones and boulders subject to severe wave action, *Lymnaea emarginata*, *L. cata-scopium*; (ii) sandy shore, species cast up from deeper water, *Valvata tricarinata*, *Amnicola limosa porata*, *A. walkeri*, *Musculium transversum*, *Anodonta kennicotti*, *Lampsilis siliquidea rosacea*, *L. ventricosa*; (iii) small shallow sandy bay containing very little vegetation, *Lymnaea palustris*, *Sphaerium tenue*; (iv) large marsh partly cut off from the lake proper, *Lymnaea stagnalis*, *L. palustris*, *L. dalli*, *Planorbis trivolvis*, *P. hirsutus*, *P. arcticus*, *Planorbula crassilabris*, *Physa gyrina*, *Aplexa hypnorum*, *Valvata lewisi*, *Musculium ryckholti*, *M. truncatum*, *Pisidium roperi*. Note: *Campeloma decisum* was not collected in this marsh, but occurs in the Grand Marais, a similar habitat on the shore of Lake Winnipeg about 15 miles south of Victoria Beach.

#### LAKES WITHOUT DIRECT OUTLET STREAMS

Lakes of this type are the reservoirs of inland drainage basins which have lost their connection with the sea as a result of changes in climate since the last period of glaciation. As there is no outlet stream to carry off the mineral salts leached from the soils of the basin and carried into the lake by its tributary streams, and the channels of underground drainage are insufficient to do this, these bodies of water are becoming increasingly saline. Such lakes are found in considerable numbers on the Prairie and Parkland, but it is only under special circumstances, such as are found subsequent to forest fires, that they occur in the forested region.

Similar lakes are found in northern Asia, and the mollusca which in that region have the greatest tolerance of saline conditions are *Lymnaea palustris saridalensis*, *L. palustris kazakensis*, and *Planorbis planorbis*.

#### Examples

1. Little Quill Lake, Sask. Total salt content in 1928, 9688 parts per million. Water analysis given by Mozley (25). *Lymnaea palustris*.
2. Lake Lenore, Sask. Total salt content in 1928, 3936 p.p.m. Water analysis given by Mozley (30). *Lymnaea palustris*, *Planorbis arcticus*.

#### INTERMITTENT STREAMS

Many of the smaller channels of drainage in this region contain no water during the period of midsummer drought, and this, coupled with the great force of flood waters when the snow melts in spring, has an unfavorable effect upon the molluscan fauna. In some instances however, where the inclination of the bed of the intermittent stream is slight, so that the freshet has not too

great a scouring effect, a few species find suitable conditions. Where this is so, large numbers of individuals may occur.

#### Examples

1. A small intermittent stream draining a group of poplar groves near Birtle, Man. *Planorbis umbilicatellus*.
2. Paskwegin Brook, a tributary of Little Quill Lake, Sask. *Lymnaea palustris*.
3. Catfish Creek, near Winnipeg, Man. *Lymnaea palustris*, *Physa gyrina hildrethiana*.
4. A shallow ditch near Mile 69, G.W.W.D. Ry., Man. *Lymnaea palustris*, *L. obrussa exigua*, *Sphaerium occidentale*.
5. A small intermittent stream near Lanigan, Sask. *Lymnaea palustris*, *L. caperata*, *Aplexa hypnorum*.

#### PERMANENT STREAMS OF THE NON-MOUNTAINOUS REGION

There is great diversity in the conditions found in the permanent streams of this area, and hence there are corresponding diversities in the molluscan fauna. It is therefore difficult to give a brief description which will apply to them all. An important characteristic of all, however, is that as they flow through fairly level country the force of the current is only moderate. In many instances this permits the growth of aquatic plants on the bottom, and the development of small marshes along the banks. Under these conditions numerous species of molluscs are to be found. The commonest forms on the Prairie and Parkland are *Lymnaea stagnalis jugularis*, *Planorbis trivolvis*, and *Lampsilis siliquoidea rosacea*. *Lasmigona complanata katherinae* is abundant in certain streams. In the forested area *Lampsilis superiorensis* is often the predominant species.

1. In pool just below rapids on a stream entering Lake Nipigon, Ont., at about N. Lat. 49° 25', W. Long. 80° 8', near Macdiarmid, Ont. *Physa ancillaria*.
2. Eyehill Creek, near Yonker, Sask. *Lymnaea stagnalis jugularis*, *Planorbis trivolvis*, *Aplexa hypnorum*.
3. Clair Brook, a tributary of Little Quill Lake, Sask. *Lymnaea palustris*, *Planorbis trivolvis*, *P. arcticus*, *Aplexa hypnorum*.
4. A small brook running into the Grand Marais, Man. Examined at a point 2 miles east of Balsam Bay. *Lymnaea palustris*, *L. obrussa exigua*, *Planorbis arcticus*, *Planorbula crassilabris*, *Physa gyrina*.
5. Muckle Creek, near Clandeboye, Man. *Lymnaea stagnalis jugularis*, *L. palustris*, *Planorbis arcticus*, *Physa integra*, *Valvata tricarinata*.
6. La Salle River, near St. Norbert, Man. *Lymnaea stagnalis jugularis*, *L. obrussa exigua*, *Planorbis arcticus*, *Valvata tricarinata*, *Amnicola* sp.
7. Jackfish Creek, east of Balsam Bay, Man. *Lymnaea stagnalis jugularis*, *L. palustris*, *Planorbis arcticus*, *P. hirsutus*.

8. Whiteshell River, below Cross Lake, Man. *Lymnaea stagnalis jugularis*, *L. megasoma*, *L. lanceata*, *Planorbis exacuus*, *P. hirsutus*, *Aplexa hypnorum*, *Campeloma decisum*.
9. Birdtail Creek, near Birtle, Man. (i) On bottom of stream, *Planorbis antrosus*, *Ferrissia rivularis*, *Physa integra*, *Lasmigona complanata katherinae*, *Lampsilis siliquoidea rosacea*, *Anodonta grandis footiana*, *Strophitus rugosus*; (ii) in small marshy areas along the banks of the stream, *Lymnaea palustris*, *L. umbilicata*, *L. parva sterkii*.
10. A backwater of the Winnipeg River, near Minaki, Ont. *Lymnaea stagnalis jugularis*, *L. emarginata canadensis*, *Planorbis corpulentus*, *P. campanulatus wisconsinensis*, *P. exacuus*, *P. arcticus*, *Physa ancillaria*, *Sphaerium crassum*, *Lasmigona complanata katherinae*, *Lampsilis superiorensis*, *Anodonta kennicotti*.
11. Red River, near Winnipeg, Man. *Lasmigona complanata katherinae*, *Lampsilis siliquoidea rosacea*, *L. ventricosa*, *Ligumia recta latissima*, *Proptera alata megaptera*, *Anodonta grandis footiana*, *Strophitus rugosus*, *Ambelma costata*.

#### STREAMS OF THE ROCKY MOUNTAIN REGION

It has been pointed out in a previous paper (Mozley (28)) that the conditions of existence in the rivers and brooks of the Rocky Mountain region are not favorable for mollusca. The reasons for this are intimately connected with the climate and topography of the region, and especially with the irregularity of flow in most of the streams, their low temperature, poverty as a nutrient medium, and the high inclination of their beds. Fresh-water mollusca have been found in only one stream in this region, viz., Caledonia Creek, near Jasper, Alta. Conditions in this stream are somewhat unusual for this mountainous part of Canada. Caledonia Creek drains a small lake, which acts as a reservoir, settling basin, and site for food production. *Valvata lewisi* and *Pisidium variabile brevius* were found in the upper part of this stream in considerable numbers.

#### The Geographical Affinities of the Fauna

The molluscan fauna of the northern part of North America has much in common with that of northern Eurasia. European elements, as distinct from Eurasian, are absent. The fauna of sub-arctic Canada is less rich than that of the more southerly parts of North America, but nevertheless has certain positive characteristics of its own.

There are three geographical elements in this fauna, namely:

(i) A group of circumboreal species, viz., *Lymnaea stagnalis*, *L. palustris*, *Planorbis crista*, *P. hirsutus* (= *albus*), *Aplexa hypnorum* and probably one or more of the Sphaeriidae. In addition, there is one North American species, *Planorbis trivolvis*, which has been found as a fossil in far northeastern Siberia (see Mozley (33)).

(ii) A large number of strictly North American species, the great majority of which are characteristic of the Mississippi drainage, or at least are found

abundantly in that region. An exception to this general rule is *Anodonta marginata* which has long been regarded as characteristic of the Atlantic coast drainage, but is now known to occur in certain parts of the Mississippi drainage.

(iii) A group of species and varieties which is characteristic of sub-arctic Canada, being either wholly confined to it, or else found most commonly within its boundaries. This element includes the following species, *Lymnaea palustris castorensis*, *L. hedleyi*, *L. preblei*, *L. randolphi*, *L. atkaensis*, *Planorbis corpulentus*, *P. corpulentus multicostatum*, *Planorbula campestris*, *Amnicola winkleyi mozleyi*, *Anodonta kennicotti*, *Lampsilis superiorenensis*, and *Lasmigona complanata katherinae*.

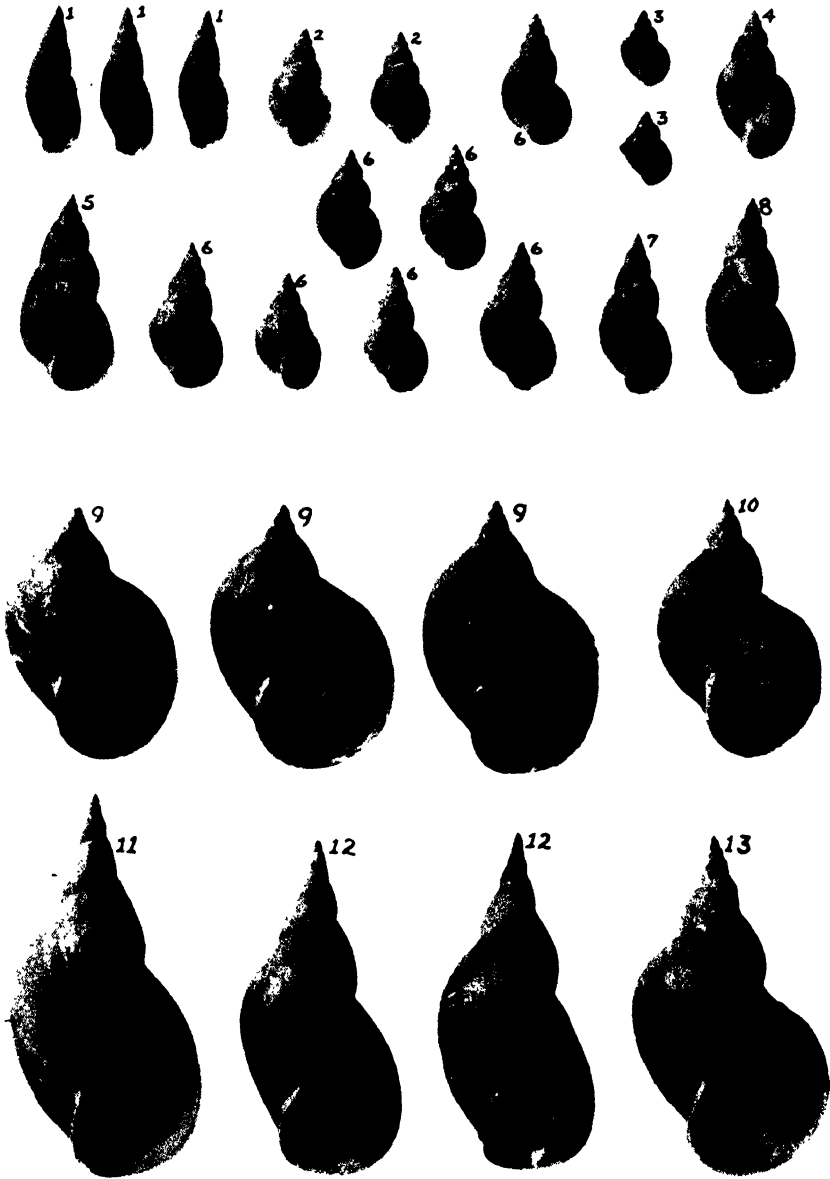
The explanation of the close relationship of the molluscan fauna of sub-arctic Canada with that of the Mississippi drainage lies in the history of the region. By far the greater part of northern North America has been subject to severe glaciation within recent times. The preglacial fauna of this region was either exterminated or driven southward before the ice sheet. After the retreat of the ice the newly exposed territory was repopulated by plants and animals which migrated northwards from the interior of the continent. General geographical factors tended to exclude the possibility of an extensive faunal migration from the Atlantic and Pacific coast regions. This was particularly true of the aquatic organisms, there being direct drainage connections between central Canada and the Mississippi River system, and little or no connection with the Atlantic and Pacific drainages. The aquatic animals which migrated into this region from the south were subsequently trapped in the northern drainage, and thus lost all possibility of interbreeding with the southern fauna. To this separation is probably due, at least in part, the continuance of the faunistic peculiarities of the northern drainage.

The total number of species and varieties of fresh-water mollusca known to inhabit sub-arctic Canada is 111. In northern Asia, apart from Lake Baikal, there are only 50 species and varieties of these animals (Mozley (33)). As both these faunas have been examined by the same investigator there is some reason for believing that the totals are comparable.

In several groups of these fresh-water mollusca there appears to have been a much greater degree of speciation in North America than in northern Asia. The Lymnaeidae of the two areas afford an example. In sub-arctic Canada, there are 26 species and varieties belonging to this family, whereas in Siberia there are only 12. The distinctly greater degree of specialization for life in certain types of habitat (e.g., temporary ponds, see Mozley (33)) in North America as compared with northern Asia may possibly be a related fact. In any event it is reasonably certain that even though the climate and general types of natural regions in the two territories are similar, there is much greater diversity of fresh-water molluscs in the Canadian fauna than in that of Siberia.

The reason may lie partly in the somewhat richer source of supply which was available in Canada, and the greater facility of migration from south to north in North America, than from west to east in Eurasia. Another important factor may have been the greater variety of suitable aquatic habitats

PLATE I



1. *Lymnaea lanceata* (Gould); Canyon Lake, Ont. 2. *Lymnaea johnsoni* (Baker); Yoho Park. 3. *Lymnaea hedleyi* Baker; Fraser River, Lucerne, B.C. 4. *Lymnaea yukonensis* Baker; Chena, Alaska. 5. *Lymnaea palustris* (Müller); pond on Duck Mountain, near Madge Lake, Sask. 6. *Lymnaea traskii* (Tryon); 1 mile west of head of Brazeau Lake, Alta. 7. *Lymnaea palustris*; Paskwegin Brook, Sask. 8. Same as 5; Birtle, Man. 9. *Lymnaea stagnalis sanctaemariae* Walker; Lake Brereton, Man. 10. Same; St. Mary's River, Sault Ste. Marie. (Bryant Walker coll.). 11. *Lymnaea stagnalis jugularis* Say; Long Lac, Ont. 12. Same; Mile 237, Hudson Bay Railway. 13. Same; Wintering Lake, H.B. Ry.



which is available in Canada for settlement by molluscs. Only the northern part of Siberia was glaciated during the Pleistocene period, whereas very nearly the whole of sub-arctic Canada was covered by ice. This has resulted in a somewhat more diversified landscape in Canada as far as the aquatic habitats are concerned. In other words, the ponds, lakes and streams of Canada offer a wider range of habitats than do those of Siberia. The general impression which is gained in travelling through northern Asia is that the landscape, particularly in the south, is more mature than that in Canada, and that the climate for some time past may have been less moist. It should be noted, however, that this conclusion may have been influenced to some extent by the fact that the studies in Siberia were carried out during a period of drought. These differences between the Canadian and Siberian landscapes, if true, might be interpreted as having some effect upon the fauna, in that new species and varieties as they arose would have less chance of finding unoccupied habitats which were suited to their special requirements. This can hardly be considered to be the sole explanation of the condition described, but it is probably an important contributing factor.

A somewhat similar conclusion has been reached by Baker (4) regarding the postglacial molluscan fauna of North America, who states that, "Previous to the Glacial Period the country had been reduced to base-level and probably few lakes existed, the physiography being one of rivers with dendritic form of drainage, like the driftless area in Wisconsin today. After the last invasion, the Wisconsin, the country was greatly changed; in place of rivers there were lakes, swamps, and sluggish rivers. The fauna reacted to this change to such an extent that where previously there had been but one or two varieties in a species, as many as ten developed which were peculiar to the newly glaciated country. Many entirely new species were evolved which have not occurred in any glacial deposits yet examined. The change affected some species more than others, but all have been affected to a noteworthy degree."

Some exception might reasonably be taken to the wording of this statement, but there is little doubt regarding the facts upon which the interpretation is based. On the whole, the conclusion, in so far as it relates to the existence of some relationship between physiography and speciation, appears to be a reasonable one.

### Acknowledgment

Owing to the fact that the author is abroad, the galley proof of this paper has been corrected by Mr. A. LaRocque of the Geological Survey, Ottawa, Ontario.

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## EFFECT OF PRECOOLING AND RATE OF FREEZING ON THE QUALITY OF DRESSED POULTRY<sup>1</sup>

By L. SAIR<sup>2</sup> AND W. H. COOK<sup>3</sup>

### Abstract

The rate at which poultry is frozen has been shown to have no effect on the number of bacteria present, and little, if any, effect on the extent of surface desiccation or development of visceral taint. The development of taint appears to depend primarily on the period during which the product is held at temperatures above the freezing point, and little advantage is gained by freezing promptly after slaughter, since taint development occurs during thawing.

A quantitative study of the amount of fluid exuded (drip) after freezing and thawing whole birds shows that, regardless of the rate of freezing, the whole bird does not drip. Freezing does change the condition of the water in the muscle, however, since drip can be obtained from minced meat after freezing. If minced meat is frozen within 3 hours of slaughter, the amount of drip is somewhat variable but apparently independent of the rate of freezing. If the birds are stored for 24 hours or more at 0° C., prior to freezing, the typical curved relation between the amount of drip and the freezing rate is obtained, the drip decreasing as the freezing rate increases.

Using a constant rate of freezing (2.5 hours to pass from 0° to -5° C.), the amount of drip decreases as the storage time prior to freezing is increased. During storage at 0° C., the greatest decrease occurs during the first day, but continues for periods up to 2 weeks. At 10° C., little decrease occurs during the first 5 days, after which it decreases slowly until the product spoils. The amount of drip obtained at a given rate of freezing appears to be proportional to the amount of fluid obtained from the unfrozen material, showing that the drip is determined by the condition of the water in the original minced muscle. There were some indications that the state of the water in the tissue was partly determined by the pH, but the results were not conclusive.

### Introduction

This investigation was undertaken to determine the effect of the rate of freezing on the quality of dressed poultry to be stored in the frozen state. When these experiments were under way it was found that the effect of the rate of freezing was conditioned by the treatment which the product received before freezing. The scope of the study was therefore enlarged to include the effect of precooling and storage prior to freezing.

It has been shown (2, 19, 20) that when fish and beef are frozen slowly in air, they exude a certain amount of fluid or "drip" when thawed. In addition to the quantitative losses, the eating quality also suffers, presumably due to the loss of certain of the flavor constituents (19, 21). The cause of drip is

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generally attributed to the formation of large ice crystals during slow-freezing (14, 17, 20), which exert a mechanical force (4) that may rupture the cells (11, 19) or prevent the cell constituents from reabsorbing the moisture (21), either because the rate of thawing exceeds the rate of diffusion back into the cells (19), or because the proteins are denatured by the processes involved in slow-freezing (5, 12, 16). Whether drip results from only one or a combination of these possible effects is not known, but it has been found (19, 20) that rapid freezing reduces both the size of the ice crystals and the amount of drip obtained when the product is thawed.

By analogy, it would appear that all meats should drip when thawed after slow-freezing. This does not appear to be the case, since slowly frozen pork and mutton (2, 19) evidently do not drip to any extent when thawed. The claimed superiority of quick-frozen poultry (10) appears to be based on the assumption that it is affected by freezing in a manner similar to beef and fish. On the other hand, it appears to be doubtful whether slowly frozen poultry does drip (10), but no direct evidence is available. Other investigations (13, 18) indicate that slow-freezing does not affect the eating quality. Further advantages have been claimed for the quick-freezing of poultry. These include: a reduction in the number of bacteria (7); a reduced development of visceral taint (10); and superior bloom and appearance, since the rate of surface desiccation is reduced (10, 22).

Most of these claims are based on the results of practice rather than experiment, and although their validity is not questioned, it seems probable that the commercial operator interprets "quick-freezing" in terms of a whole series of processes, rather than as the strict rate of freezing which the term implies. For instance, where quick-freezing is practised, the period between slaughter and freezing may be reduced, the birds eviscerated, and the entire marketing and distributing chain improved. The over-all process may therefore result in a superior product which cannot be attributed entirely to the rapid rate of freezing.

The major part of this investigation was devoted to the question of drip in frozen poultry. The effect of the rate of freezing on bacterial count, visceral taint, and surface desiccation was investigated in a preliminary way, and the results of the tests will be reported first.

### Number of Bacteria

Haines (6) has shown that the temperature of freezing between  $-5^{\circ}$  and  $-70^{\circ}$  C., *i.e.*, rate, has little effect on the ratio of viable organisms before and after freezing. On the other hand, Heitz and Swenson (7) report that the number of bacteria on slow-frozen ducks is 1000 times greater than that on the quick-frozen product. Although this difference is attributed to quick-freezing, it appears that the two lots of ducks used were not subjected to exactly the same treatment, and the observed differences may therefore be due to other factors than the rate of freezing.

In order to provide material for these tests, chickens were stored for 3 weeks at 0° C. (32° F.). Since bacterial development is greatest on the surface, the meat was removed, minced, mixed, and allowed to stand overnight at room temperature. It was then placed in small metal containers, frozen at various rates, and stored for 2 days at -40° C. (-40° F.). The samples were thawed by placing them in air at 15° C. (60° F.) for 4 hours. They were then ground with sterile sand and extracted with sterile water. Counts were made after plating appropriate dilutions on beef-extract agar and incubating for 48 hours at 25° C. A second experiment was conducted in a similar manner except that the chicken meat was transferred directly from the room at 0° C. (32° F.) to the freezing chambers, and ground in the frozen condition.

The results obtained are given in Table I, from which it is evident that the rate of freezing has little, if any, effect on the number of bacteria present. This finding appears to be in agreement with Haines (6). In practice, the quick-frozen product is usually frozen shortly after slaughter, whereas the slow-frozen product is frequently stored at a temperature near

TABLE I  
EFFECT OF RATE OF FREEZING ON NUMBER OF BACTERIA  
IN MINCED CHICKEN MEAT

Rate of freezing (0° C. to -5° C.), hr.	Bacterial count per gm. of meat, log <sub>10</sub>	
	Exp. 1	Exp. 2
Check (unfrozen)	8.60	9.70
0.5	8.84	9.48
4.0	8.46	9.65
24.0	8.62	9.56

the freezing point for several days before freezing. Since bacterial growth at 32° F. appears to be relatively slow (8) it is difficult to account for the results of Heitz and Swenson (7), even on the basis of somewhat delayed freezing of the slow-frozen product. It appears that their material must have been exposed to temperatures above the freezing point for a considerable period, or else the material was not comparable in other ways. It is concluded from the results of the present investigation that quick-freezing confers no advantage over slow-freezing, from the standpoint of the bacterial numbers in properly handled poultry.

### Surface Desiccation

Tests of the effect of the rate of freezing on surface desiccation were restricted to a study of the loss of moisture during freezing, and the effect of various other storage conditions will be described in another paper (1). The initial attempt to obtain quantitative information on this subject was made by determining the moisture content of the skin before and after freezing. This failed, owing to the marked variation in the moisture content of chicken skin, and the difficulty of obtaining a representative sample. The method finally adopted was to weigh the bird to an accuracy of 0.5 gm. before and after freezing. This gives the over-all shrinkage or loss during the freezing process, and possibly exaggerates the amount of moisture lost by the skin only.

Six birds, having an average weight of 6 lb., were precooled to 0° C. (32° F.). Three of these were frozen by hanging in a room at -40° C. (-40° F.), and three by hanging in a room at -13° C. (+7° F.). The time required for the centre of the birds to reach a temperature of 20° F. was 1.5 and 16 hours respectively. The freezing process resulted in a loss of 3, 2, and 2 gm. for the quick-, and 4, 3, and 3 gm. for the slow-frozen birds. This corresponds to a mean over-all loss of 0.08% and 0.12% respectively, or a difference of 0.04% between the two rates of freezing. Even if this loss came entirely from the skin, it could hardly be expected to render the product at all independent of the storage conditions under which it is subsequently kept, since these will be shown (1) to have a marked effect on the rate of development of surface marking. In commercial practice the birds would be frozen after packaging, and although this would reduce the rate of freezing, it would also reduce the shrinkage to less than the values reported above.

### Development of Visceral Taint

Mandeville (10) states that one of the main advantages of quick-freezing is to prevent the development of off-flavors which take place after death; but experimental evidence to support this statement is lacking. Commercially, quick-freezing is frequently practised on eviscerated poultry, and the benefits of these two distinct processes, evisceration and quick-freezing, may have become confused. Nevertheless, it is possible that the rate of freezing may affect the development or transmission of taint from the viscera or other regions.

There are no adequate methods for measuring the amount of taint given off by a particular organ or region during freezing, or its effect on the odor or flavor of the meat after transmission. In these tests the whole viscera were used and an attempt made to determine: the effect of prompt *versus* delayed freezing; the effect of both freezing and thawing separately and combined; and the effect of storage at 0° C. (32° F.) without freezing. The method used was based on the "enfleurage" method (15), or the absorption of the odor by a fatty substance. The general procedure consisted of removing the viscera, wrapping them in blotting paper supported by a cotton bag, and placing the whole in quart sealers previously coated thinly on the inner surface with Crisco. The sealers were stoppered and treated in accordance with the requirements of a particular experiment. Quick-freezing was accomplished by immersing the sealer in an ethylene glycol water bath at -33° C. (-28° F.), and slow-freezing by insulating the sealer with about one inch of cotton wool and placing in air at -13° C. (7° F.). The exact time required for freezing under these conditions was not determined. Thawing was accomplished in all cases by placing the sealers in a cabinet at 10° C. (50° F.) for two days. After thawing, the viscera were removed, and the sealer was stoppered tightly and left at room temperature for one day. Five persons then estimated the intensity of the odor in accordance with the following score card: 0—no foreign odor distinguishable; 1—ethereal but not distasteful; 2—unpleasant;

and 3—intense and disagreeable. The values reported in Table II are the average of the five scores made in duplicate.

The summarized results given in Table II show some irregularity, which is to be expected in subjective tests of this sort, but they are reasonably consistent. It appears that tainting substances do develop in the viscera during slow-freezing (Exp. I), thawing (Exp. II), precooling prior to freezing (Exp. III), and storage in the unfrozen state (Exp. IV). The advantage of quick-freezing the warm viscera, observed in the first test, is not shown by the second. The elapsed time before freezing appears to be more important than the rate of freezing. The taint apparently develops largely during the thawing process. No evidence of free drip was obtained at any rate of freezing. These results indicate that visceral taint is entirely a question of the period during which the product is held above the freezing point. Evisceration rather than prompt or quick-freezing appears to be the obvious solution for this difficulty.

TABLE II  
DEVELOPMENT OF VISCERAL TAIN

Exp. No.	Treatment of viscera	Processes involved in test	Intensity of odor* (average score of 5 persons)	
			Slow-frozen	Quick-frozen
1	Not precooled prior to freezing	Freezing, 2 days' storage at $-28^{\circ}\text{C}.$ , and thawing.	2.4	0.7
2	Not precooled prior to freezing	{Freezing only. {Thawing only.	0.6 2.6	0.4 1.4
3	Precooled 24 hr. before removal from bird	Freezing, 2 days' storage at $-28^{\circ}\text{C}.$ , and thawing.	1.7	1.4
4	Not precooled prior to placing in jar	Not frozen, stored for 6 days at $0^{\circ}\text{C}.$	2.8	

\* Maximum intensity score—3.0.

It is difficult to interpret the taint intensities observed in terms of flavor of the meat. Poultry stored at  $0^{\circ}\text{C}.$  ( $32^{\circ}\text{F}.$ ) for six days, or even longer, is generally considered of good eating quality, but this treatment resulted in the most intense odor recorded. Possibly, more taint would be developed by the viscera during storage in the frozen state, a condition not included in these studies. Since the amount of taint developed during frozen storage would be more likely to depend on the temperature and other storage conditions than on the rate of freezing, this fact would appear to furnish a further reason for evisceration.

### Exudation of Tissue Fluids (Drip)

#### METHOD

It is convenient at this point to present the method employed for determining the amount of fluid exuded after freezing, *i.e.*, drip. Essentially, it

consisted of a slight modification of that used by other investigators (2, 17) for determining the drip in beef and fish. Other methods were also studied for purposes of comparison and will be discussed later in relation to the results obtained.

A sufficient quantity of meat for the experiment in question was obtained from several chickens, ground, and thoroughly mixed. Samples of about 100 gm. were taken, placed in tared metal dishes, about 3 in. in diameter and 1 in. deep, having removable tops and bottoms. The samples were weighed accurately, frozen at the desired rate, stored at the freezing temperature for at least 2 days, and then thawed by placing them in a tight-fitting cylinder, jacketed with water at 15° C. Thawing by this technique required about 4 hours. The drip was then determined by replacing the tops and bottoms of the cans with several layers of blotting paper, the layers being held in place by a weight equivalent to 1 gm. per sq. in., and allowing them to stand for 20 hours, at 0° C. The blotters were removed, the sample reweighed and the loss computed. The loss in weight of an unfrozen control sample was similarly determined and subtracted from that of the frozen sample to obtain the quantity reported as net drip. Usually triplicate, and frequently quadruplicate samples were used for each test and control measurement.

In order to obtain an estimate of the accuracy of this method, the standard error of the mean of triplicate samples was computed for 20 determinations chosen at random. It was found to be  $\pm 0.53\%$ . Similar calculations for the controls, which had a lower total loss of weight, gave an error of  $\pm 0.20\%$ . The standard error of the difference, *i.e.*, net drip, would therefore be  $\pm 0.57\%$ , and the 5% point approximately 1.1%. Differences in net drip greater than 1.1% can therefore be considered significant.

#### DRIP IN RELATION TO RATE OF FREEZING

To determine the amount of drip obtained at different rates of freezing, preliminary experiments were made on: eviscerated whole chickens; skinned half chickens; meat cut into approximately 1-in. cubes; minced dark meat; and minced light meat. The birds frozen whole were eviscerated to avoid the possible accumulation of drip in the body cavity, and the skin was removed from the half-birds used in the second experiment to prevent it from retaining any drip. The method employed in these instances was to place the birds in moisture-tight cans, freeze them at the desired rate, thaw at 10° C. for 2 days in a manner which permitted any liquid to accumulate in the bottom of the can, and then measure the amount of liquid. This procedure gives an estimate of the free drip plus any liquid that may have evaporated from the birds and condensed on the can during the freezing process. The cut and minced meat samples were tested by the method already described.

The results obtained are reported in Table III. It is evident that the amount of drip obtained from whole birds is negligible regardless of the rate of freezing. Removal of the skin, cutting, and mincing the meat progressively

TABLE III  
EFFECT OF RATE OF FREEZING ON WHOLE BIRDS, AND CUT AND MINCED POULTRY MEAT

Material		Freezing rate: time to pass from 0° C. to -5° C. (32° F. to 21° F.)		
		1 hr.	8 hr.	18 hr.
Whole birds (eviscerated)*	Drip %	0.05	—	0.15
Half birds (skinned)*	Drip %	1.0	—	1.9
Cut meat, (1-cm. cubes approx.)†	Drip %	1.4	2.3	2.5
Dark meat (minced)†	Drip %	2.0	6.5	6.8
Light meat (minced)†	Drip %	2.9	7.4	8.7

\* Drip estimated from weight of free liquid obtained.

† Drip estimated from differential weight loss of frozen and unfrozen samples after absorption of liquid with blotting paper.

increase the amount of drip obtained, and on the minced tissue the effect of different freezing rates can easily be ascertained. Although all the methods indicate that the amount of drip decreases as the freezing rate increases, the observed differences in the amount of free drip obtained from the whole, and half chickens could be attributed to experimental error. The observed differences, using cut meat and absorption with blotting paper, are too small to establish any definite relation between drip and freezing rate, so that minced meat was used for subsequent measurements. The results in Table III show that light meat yields more drip than dark meat, but as the difference was not great, both kinds were mixed and ground together in preparing material for later work.

Before initiating the main series of investigations, a number of chickens of different weight (age) from different sources were studied. All of these yielded essentially the same amount of drip at the same freezing rate. It was found, however, that the period allowed for precooling between slaughter and freezing did affect the quantity of drip obtained. In consequence, two post-slaughter treatments were used; in one, the meat was minced and placed in the freezer within 3 hours of slaughter, and in the other, the birds were pre-cooled for 24 hours at 0° C. (32° F.) before being cut up, minced and frozen. Unless otherwise stated, the birds were all starved for 24 hours prior to slaughter.

The effect of various rates of freezing on drip formation is given in Fig. 1 for minced meat frozen within 3 hours of slaughter, and for minced and cut meat after 24 hours precooling. The rate of freezing is expressed as the time required for the product to pass from 0° C. (32° F.) to -5° C. (21° F.). As indicated by the results given in Table III, cut meat yields less drip than comparable minced samples. The curves from the cut and minced meat, however, have the same general form. After precooling the birds for 24 hours, the quantity of drip obtained from minced meat decreases with the rate of freezing. The quantity of drip increases quite rapidly up to about a 4-hour freezing period, after which it increases relatively slowly so that longer freezing

times give about the same amount of drip. This is the typical relation which other investigators (11, 17) have found with beef and fish. It is evident from the curve that a freezing time of about an hour is required if the quantity of drip is to be reduced to half that obtained by slow-freezing.

The amount of drip obtained from minced chicken meat frozen within three hours of slaughter was somewhat variable, but was always equal to, or greater than, the maximum quantity obtained from birds that had hung overnight (Fig. 1). The fact that large quantities of drip were obtained from quick-frozen material of this sort indicates that some post-mortem change takes place during precooling, which tends to reduce the quantity of drip obtained. The relatively constant quantity of drip obtained at all freezing rates may, therefore, be the result of two opposing tendencies, slow-freezing tending to increase drip, but allowing the post-mortem changes which reduce it to take place and *vice versa*.

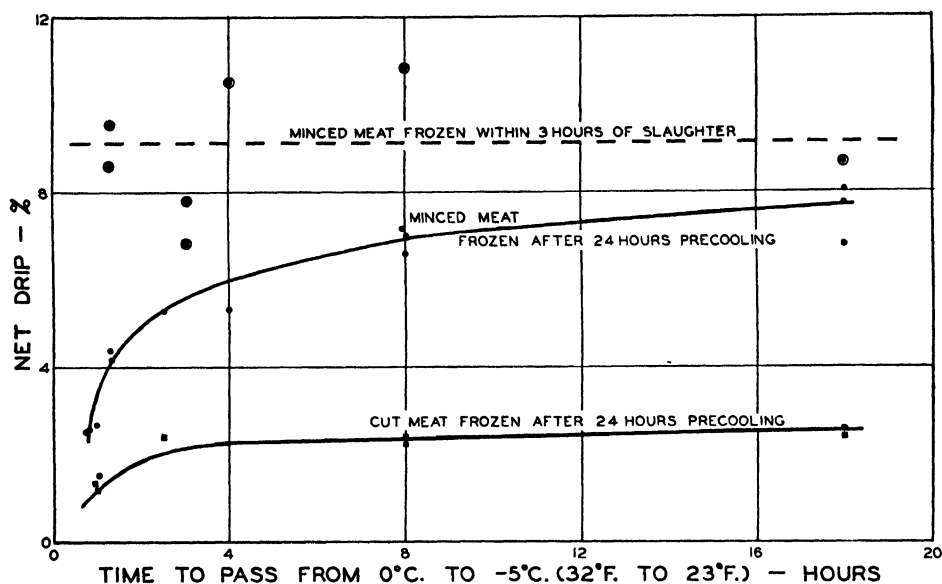


FIG. 1. Drip in relation to rate of freezing.

It should be noted that although the drip from minced meat is relatively high and is related to the rate of freezing, whole chickens show negligible drip at any freezing rate. This fact brings up the question as to the nature of the deterioration, if any, that occurs when chicken meat is frozen. In other words, does the change in the water relations caused by freezing, as evidenced by the drip in the minced meat experiments, cause deterioration, or must this drip escape from the tissues before any effect is noted? If the latter view is accepted, then the rate of freezing has no effect on the quality of poultry since no fluids escape from the whole carcass.

## DRIP IN RELATION TO STORAGE BEFORE FREEZING

Further investigations were undertaken to determine the effect on drip of storage prior to freezing, since investigations (4) on beef indicate that the period between slaughter and commencement of freezing had no definite effect on the quantity of drip obtained. One rate of freezing was used throughout, *i.e.*, 2.5 hours to pass from  $0^{\circ}\text{C.}$  to  $-5^{\circ}\text{C.}$ , the product being stored at  $0^{\circ}\text{C.}$  ( $32^{\circ}\text{F.}$ ) and  $10^{\circ}\text{C.}$  ( $50^{\circ}\text{F.}$ ) for various periods prior to freezing. The higher storage temperature was used to determine whether the observed changes occurred more rapidly as the temperature increased.

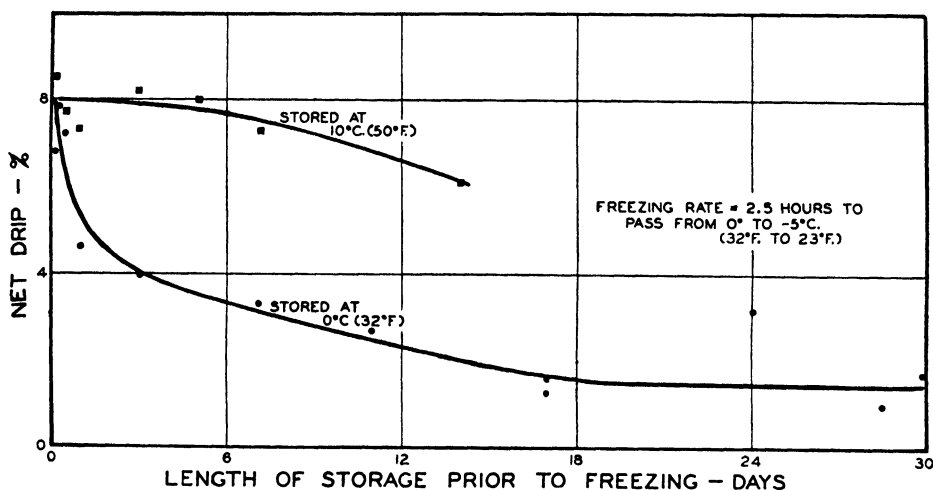


FIG. 2. *Drip in relation to storage before freezing.*

The results obtained were used to construct the curves in Fig. 2. It is evident that storage at  $0^{\circ}\text{C.}$  prior to freezing decreases markedly the amount of drip during the first 24 hours, after which there is a slow decrease during 18 days' storage. Beyond that time the drip is more variable, probably due to incipient putrefaction, but appears to remain reasonably constant throughout the storage of the product at this temperature. This change in the quantity of drip obtained during storage cannot be attributed to evaporation since all birds were stored in closed containers at 100% relative humidity. The birds stored at  $10^{\circ}\text{C.}$  ( $50^{\circ}\text{F.}$ ) showed a constant drip of about 8% for the first 6 days after which it fell off slightly until the 14th day when the test was terminated owing to putrefaction of the meat.

These experiments show that certain rigor and post-rigor changes occur during storage at  $0^{\circ}\text{C.}$  which tend to reduce the drip obtained after freezing. These changes do not take place to any extent at  $10^{\circ}\text{C.}$  It is possible that two types of change can take place: one during rigor, which causes a rapid increase in the water-retaining capacity of the tissue; and another which acts in the same direction but more slowly, as indicated in the lower curve. If this explanation is correct, the difference between the curves obtained at

0° C. and 10° C. is that the first change does not occur at the higher temperature.

These results, which are typical of many more experiments, show that the amount of drip obtained depends on the initial condition of the tissues as well as on the rate of freezing. Further evidence favoring this view arose from the observation that when the drip in the unfrozen control was high, the total drip in the frozen sample at a given rate of freezing was also high, and *vice versa*. The difference between these two quantities, or the net drip, usually showed the same behavior. In order to confirm this observation, the percentage of total drip obtained from a number of frozen samples was plotted against the drip obtained from the corresponding unfrozen controls. The resulting graph is shown in Fig. 3. It is evident that the amount of drip

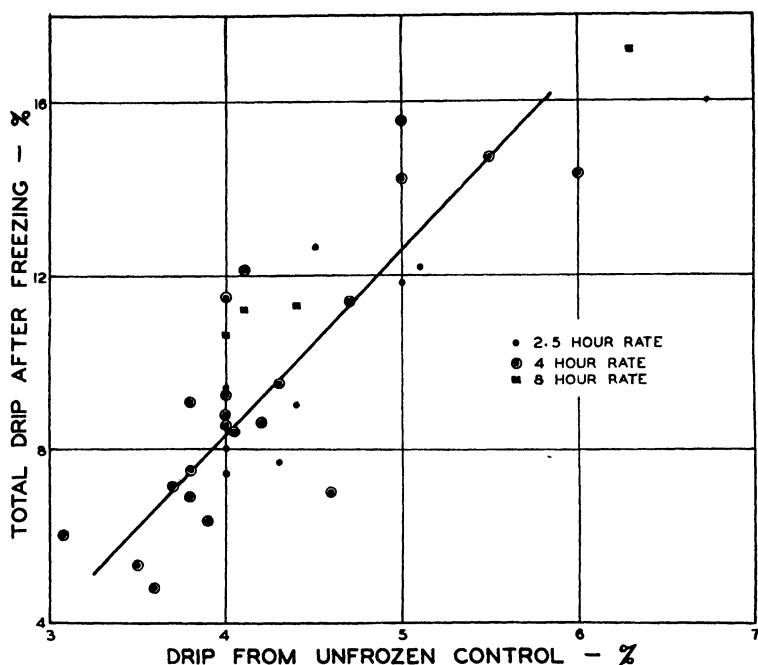


FIG. 3. Drip in relation to condition of muscle.

obtained after freezing depends on the tenacity with which it is held in the original unfrozen sample. Storage prior to freezing causes the tissues to retain their moisture more firmly, but other factors may also affect the condition, and the nature of the changes that take place is still obscure.

#### DRIP IN RELATION TO CONDITION OF MUSCLE TISSUE

In an attempt to determine the nature of the conditions governing the state of water in muscle tissue, and consequently the amount of drip, the first experiment undertaken was an analysis of the drip. The drip was obtained from approximately 500-gm. samples of meat, by subjecting the thawed

samples, contained in narrow mouthed percolators, to a pressure of 0.35 lb. per sq. in. for 3 days at 0° C. (32° F.). A slight suction was applied from time to time to remove the free liquid which collected in the voids. Samples of chicken meat were obtained immediately after slaughter, and also from birds stored 24 hours before mincing and freezing. Both tests included an unfrozen control and samples frozen at two rates. The quantity of drip thus obtained provided material for analyses and also established that there was a marked relation between the amount of free drip from minced tissue and the quantity obtained by the blotting paper method. After three days, when the last of the drip had been removed, 500 ml. of 2% KCl was added to the percolators, allowed to stand for 24 hr., and then drained off for analysis.

The results of the analysis performed on the exuded fluid and percolate from each sample are reported in Table IV. It is evident, in spite of the fact that the amount of drip obtained from the different samples varied in accordance with the treatment and freezing rate, that the composition of these fluids, with respect to the analyses performed, was essentially the same for the material frozen immediately or after 24 hours, and for the unfrozen or frozen samples. It appears, therefore, that the fluid exuded from the tissue is of relatively constant composition regardless of the treatment, or the amount obtained. This finding is in agreement with that obtained by Empey (4) on beef.

TABLE IV  
COMPOSITION OF DRIP

Rate of freezing (time to pass from 0° C. to -5° C.), hr.	Free drip, %	Original drip					Percolates	
		pH	Total solids, %	Non-combustible solids, %	Protein nitrogen, %	Non-protein nitrogen, %	Total nitrogen, %	Organic matter, %
Frozen within 3 hr. of slaughter								
Unfrozen control	5.7	6.15	14.8	1.20	1.68	0.48	0.68	5.0
1.3	14.3	6.20	15.0	1.24	1.60	0.48	0.68	4.8
18.0	14.4	6.05	14.7	1.23	1.76	0.48	0.66	5.3
Stored for 24 hr. at 0° C. prior to freezing								
Unfrozen control	4.2	6.35	14.4	1.26	1.70	0.44	0.64	5.2
1.3	8.4	6.30	14.8	1.23	1.72	0.43	0.63	4.7
18.0	12.0	6.25	14.6	1.19	1.70	0.46	0.61	5.6

Empey found that the period between slaughter and commencement of freezing has no effect on the quantity of drip from beef. Furthermore, he states that the amount of drip is minimal in muscle tissue at about pH 6.3 and that conditions more acid than this tend to increase the quantities obtained. These results appear to be conflicting, since the formation of lactic acid in the

muscle after slaughter usually results in a gradual decrease in pH which attains finally a value considerably more acid than pH 6.3. If pH is the principal factor determining the loss of fluids, then it would appear that the drip should increase for a certain period after slaughter. Chicken meat may not be comparable with beef, but the fact that the drip definitely decreased with time from slaughter, and the apparent conflict in Empey's results, rather indicate that another factor than the pH of the tissue is involved.

TABLE V  
EFFECT OF STORAGE PERIOD BEFORE FREEZING, AND pH, ON DRIP  
Rate of freezing--4 hr. to pass from 0° to 5° C.

Test No.	Pre-slaughter treatment	Storage period at 0° C. (32° F.) prior to freezing, hr.	pH		Drip		
			When minced	When thawed	Un-frozen control, %	Total (frozen sample), %	Net %
1	Normal: birds pre-starved 24 hr. before slaughter	4	5.8	—	—	15.6	—
2		24	6.0	—	—	9.3	—
3		144	6.1	—	—	8.4	—
4	Birds pre-starved 24 hr. received injections of insulin prior to slaughter	4	6.0	—	—	11.9	—
5		24	5.8	—	—	5.2	—
6		144	6.0	—	—	4.3	—
			(Uncut meat)				
7	Normal: birds pre-starved 24 hr. before slaughter	0.5	7.0	6.3	6.0	14.3	8.3
8		24.0	5.5	5.9	—	9.0	—
9	Birds pre-starved 24 hr. received injections of sodium iodoacetate prior to slaughter	0.5	7.2	6.6	5.0	14.4	9.4
10		24.0	5.6	6.1	4.3	9.5	5.2
11	Normal: birds pre-starved 24 hr. before slaughter	24	—	5.9	4.1	12.1	8.0
12		120	—	5.8	3.7	7.0	3.3
13	Pre-starved for 24 hr. and exercised prior to slaughter	24	—	6.2	3.8	6.8	3.0
14		120	—	6.1	4.6	7.2	2.6
15	Fed until slaughter	14	—	5.9	3.8	9.1	5.3
16		120	—	5.9	3.9	6.4	2.5
17	Birds received injections of both insulin and glucose prior to slaughter	24	—	5.8	4.7	11.4	6.7
18		120	—	5.7	3.8	7.5	3.7

These considerations led to some experiments in which the pH as well as the drip was studied, after various storage periods. Preliminary measurements on the pH of minced chicken muscle indicated that it usually fell within the narrow range of 5.8 to 6.1. Attempts were therefore made to extend the range of pH values obtained. Three experiments of this sort were made.

The first two were concerned primarily with the changes in pH and drip within 24 hours after slaughter, and the third with the changes that occur after a 24-hour storage period. The initial experiment included normal, pre-starved birds, and similar birds that received 120 units of insulin (3) prior to slaughter. A comparison of the changes that occur in normal, pre-starved birds, with those that take place in similar birds injected with 0.04 gm. of sodium iodoacetate (9) prior to slaughter was made in the second experiment. The final test included the following treatments: normal, pre-starved birds; birds similarly starved but vigorously exercised for 10 min. before slaughter; birds fed until slaughter; and birds that received both intravenous glucose and subcutaneous insulin 90 min. before slaughter. All measurements on this material were made 24 hours or more after death.

The results of the experiments are given in Tables V and VI. All the pH measurements were made with a glass electrode and the values are corrected to 20° C. (5), although the majority of the observations were made at lower temperatures. Owing to the limited amount of material available for the first experiment, only the total drip from the frozen sample was tested. The results show that the pH of tissue from both the normal and insulin-treated birds was quite similar at the time of mincing, and changed very little with time of storage. On the other hand, the quantity of drip from the insulin-treated birds decreased more rapidly during storage than did that from the normal birds. This suggests a relation between carbohydrate metabolism and drip, although the pH values show no evidence of any great difference in the amount of acid produced. In the second experiment, it was found that the injection of iodoacetic acid only maintained the neutral condition for a matter of 2 hours (Table VI) or less, after which it behaved as the normal sample. Therefore, no conclusion is possible from this test, since the pH and drip values were the same from both normal and treated birds.

The final experiment involved a number of treatments, and the drip and pH (when thawed) measurements were made one and five days after slaughter. In all cases, the drip decreased during storage and the tissue tended to become slightly more acid. The pre-starved and exercised birds were the most alkaline throughout and showed the least drip. This result appears to support Empey's findings. The pH and drip for the other treatments were essentially the same throughout.

Investigations relating to pH and drip formation in chicken meat are complicated by the fact that no evidence of free drip from the whole carcass was obtained. This,

TABLE VI  
CHANGES IN pH OF CHICKEN MUSCLE DURING STORAGE  
AT 0° C.

Time from slaughter, hr.	pH of whole muscle	
	Normal birds	Birds injected with sodium iodoacetate
0.5	7.0	7.2
1.5	6.5	7.3
4.0	6.2	6.3
6.0	5.6	5.8
24.0	5.5	5.6

together with the possible variable behavior of the muscle from different chickens, led to a study of drip in relation to the effect of pH and storage on beef, pork, and mutton. The results of these investigations will be reported shortly.

It is of interest to report the observation that the pH appears to affect the color of the muscle. In these experiments, the pH range obtained in samples 24 hours after slaughter was relatively narrow, yet the more acid samples had a light pink color while the more alkaline samples had a darker appearance, tending toward a brown. This observation may be of some significance and is being studied in a more quantitative manner on other meats.

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# THE INFLUENCE OF NUTRITIONAL AND CLIMATIC FACTORS ON WOOL GROWTH AND QUALITY

## I. STATEMENT OF PROBLEM AND EXPERIMENTAL PROCEDURE<sup>1</sup>

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### Abstract

A five-year study has been conducted on the effect of humidity, temperature, date of shearing, plane of nutrition, and protein or mineral intake, on wool growth and quality. The results indicate that significant differences in wool growth and quality may be obtained by varying the shearing dates and the plane of nutrition of the ewes. Little or no effect was evident when ewes were subjected to differences in temperature or humidity, or in mineral and protein intake levels.

Following a conference in the City of Toronto in May, 1927, an Associate Committee on Wool was organized by the National Research Council of Canada, in co-operation with Canadian producers and manufacturers of wool, government departments, and agricultural colleges. It was realized that Canada depended too largely on wool from other countries and it was felt that if Canadian wool could be improved sufficiently to increase its use in domestic manufacture, it would be beneficial to all phases of the industry.

A sub-committee was formed to consider what studies might be undertaken in the field of production. As a result of a survey of certain sheep ranches in western Canada, it became evident that the greatest hope for the improvement of Canadian wool lay in breeding experiments. It was also obvious that in such experiments attention should be paid to mutton quality as well as to wool.

Based on a report of the sub-committee, two experimental breeding projects were established, one at the University of Saskatchewan, Saskatoon, and the other at Cardston, Alberta. Both were assisted financially by the National Research Council, and the experiment at Cardston was supervised by a small group of the Associate Committee on Wool.

In order to evaluate a possible improvement in wool through breeding, it was considered necessary to investigate any effect attributable to general environmental conditions. At that time little information was available concerning the relation of climate and nutrition to wool growth and quality. It was arranged that an investigation of this particular problem, under controlled conditions, should be undertaken at the University of Alberta. Studies were planned to demonstrate the effect on wool growth and quality of (i) humidity, (ii) temperature, (iii) early and late shearing, (iv) plane of nutrition, (v) protein levels, and (vi) mineral intake. Wool samples from the different groups of sheep were forwarded at regular intervals to the National Research Laboratories in Ottawa for critical analysis. For several years, entire fleeces from

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all the lots were subjected to special grading at the warehouse of the Canadian Co-operative Wool Growers, Weston, Ontario.

The investigation covered a five-year period, 1929-30 to 1933-34, and was supported financially by the National Research Council. The results, obtained at the University of Alberta, in the National Research Laboratories, and at the Weston warehouse of the Canadian Co-operative Wool Growers, will be presented in a series of three papers, of which this is the first.

### Review of Literature

One of the first published statements concerning the relation of wool growth and quality to the animal's environment was reported by Youatt (39), who stated that "about 1776 a Dr. Anderson noticed that fibre diameters were greater when grown under high temperatures." In 1806, Dr. Parry reported in the Bath Society papers, that he had observed a "sort of gross connexion between food and the quality of the fleece," but was not certain "that coarseness of wool and tendency to grow fat are connected." Further evidence of how climate and nutrition influenced the gross weight and wool quality was reported by numerous individuals, based on observations and crude experimentation. It was generally observed that a variation in wool occurred in different regions and in the same region from year to year.

Five or six German investigators have studied seasonal wool growth and have shown that practically none occurred during the last two months of the yearly production, while approximately two-thirds took place during the first six months after shearing. In studies on the monthly wool growth of Rambouillet (6), Hampshire, and Corriedale (7) ewes, Burns found that the seasonal variations were not as great as those reported by the German investigators. These three breeds produced, during the first six months after shearing, 54, 52 and 57% respectively of their yearly growth. Lush and Jones (23) report that the effect on the fleeces of very droughty seasons is greater than the influence of age. Burns (6) states that Nordmeyer secured more wool growth during those months when green feed was available, and less when the ewes were suckling lambs.

The difference from year to year at the University of Alberta, in total raw wool produced by a flock numbering about 225, has been over 300 pounds. This indicated that after prolonged winters of low temperatures the raw fleece weights were less than those clipped after moderate winter conditions.

Hardy and Tennyson (21) found that the rate of wool growth and the fineness varied throughout the year, both growth and coarseness being greatest in summer and fall, and least in mid-winter. They also found that wool production is associated with general thrift and condition of the sheep as indicated by their weight. The period of least growth occurred in ewes usually during lambing time and the 45 preceding days. Numerous experimenters have shown that wool growth and quality are affected by differences in feed supply which may be the result of natural feeding conditions or controlled feeding trials. Duerden (13) found that wool grew faster during the

summer, when the sheep were in pasture, than during the winter months. Studies by Tinley (35) indicated that thick, lush herbage tends to stimulate the production of a larger and coarser grade of wool than does the thin, sparse herbage associated with the finer grades. Smith and Hussain (32) found that growth of wool was affected by the lambing season and by periods of abundance or lack of feed. On the other hand, Fraser (17) found that wool grew at a uniform rate throughout the year when sufficient pasture was provided.

In controlled feeding trials where wide differences in the nutrition of ewes could be secured, marked variations in growth and quality of wool have been noted. Wilson (38) compared the wool production of sheep on a fattening ration with that of a second group on a sub-maintenance ration, and found differences of 41% in fibre growth, 343% in grease weights, 319% in scoured weights, 207% in fibre strength, and 26.5% in fibre diameter, in favor of the fattening ration. He concludes that a "state of semi-starvation such as thousands . . . . of sheep are subjected to on our western ranges during two to four months of the year . . . . will not produce good wool." Weber (37), and Snell (34) secured similar results, in that sheep maintained on a low plane of nutrition produced shorter and finer wool, and much less scoured wool. Mitchell (27) fed a group of sheep on a sub-maintenance diet, and although the animals were in a negative energy and nitrogen balance for over 200 days, they stored energy and nitrogen in their wool at approximately normal rates.

One authority does not believe that wool growth can be easily affected by changes in level of feeding. Joseph (22), as a result of his experiments, concluded that "in sheep of fine-wool breeding, the organs which are concerned with the secretion of the wool fibre are not easily subject to such influences as changes in the level of feeding, especially for periods of 5 or 6 months, if the sheep remain in normal health; that the quality of the wool fibre is affected not at all, and that the quantity of fibre may be modified only slightly, as long as the sheep remains in normal health." He advocated that "level of winter feeding should be based on the needs of the sheep for health and condition for withstanding rigorous winter climatic conditions rather than for wool". Similarly Cooke (8) found that wool growth is only slightly reduced when the ration is insufficient for maintenance.

Dowell and Bowstead (12) conducted a series of pregnancy experiments and concluded that "any roughage or combination of feeds that maintains the ewes in good, vigorous condition tends to produce a heavier fleece than the less satisfactory feeds." There appears to be ample proof that the plane of nutrition does affect wool growth and quality, though there is much to be learned concerning the extent and limitations of such an effect.

Since the wool fibre is chiefly protein (keratin), research workers have done considerable work to determine the effect on wool growth of feeding low and high levels of protein; or special proteins, or minerals high in sulphur. As early as 1890, Cooke and Jones (9) in Vermont fed a carbohydrate ration to one group and a nitrogenous ration to another. Their results showed that

there was no measurable difference in diameter of fibre. They pointed out, however, that the wool fibres of a few sheep that "did not do well" were "shrunk in diameter". About the same year, Craig (10) at Wisconsin conducted a similar trial and concluded that sheep fed on the nitrogenous ration clipped 2.4 pounds more unwashed wool and 0.6 pounds more washed wool than those on the carbohydrate ration. The additional weight in the unwashed wool was credited in a limited degree to the amount of yolk present.

Marston (24), feeding blood meal to increase the protein level, found that the addition of the supplement increased the raw and clean fleece weights by over 35%.

Fraser (18) reported that at the Rowett Institute "it has been found that the feeding of a relative excess of protein gives a 20% increase in crude fleece weight. Conversely the feeding of a protein-deficient ration gives a 20% decrease in fleece weight, . . . . these figures are closely reflected in the scoured fleece yields." However, in collaboration with Roberts, Fraser (20) later reported an experiment in which soybean meal was used to increase the level of protein intake. They concluded that "increasing the protein in the ration caused no significant difference between the two groups in any of the wool characteristics measured," which were dry weight, fineness, fibre length and grease. A seemingly opposite effect was later obtained by Fraser, who with Nichols (19) secured an increase in wool growth by adding carbohydrates to what they termed a balanced ration.

The protein problem has been further studied in relation to the sulphur and cystine content of feeds. When Marston and Robertson (25) found that the cystine content of wool was constant, they assumed that the quantity of cystine available in the diet must be a factor limiting the quantity of wool that can be produced. Increased wool growth was secured by Marston (26) when cystine was administered to one sheep on a low protein diet, and the response was three times greater "when cystine was injected subcutaneously so as to avoid destruction of the free amino acid by bacteria in the alimentary canal." Barritt and King (2), and later, Rimington (29 and 30) found that the cystine in wool was not constant; they suggested that probably sheep had the ability to synthesize cystine. The work of Bosman (4) also indicated the synthesis of cystine. He found that the sulphur content of wool grown during periods of adequate nutrition is higher than during periods of scarcity, and is not related to the sulphur in the diet. In his experiments, controlled feeding of maize meal (poor in cystine) for a year failed to bring about marked decrease in either fleece weights or in sulphur content of the wool. It would appear that more cystine is used in producing wool than can be accounted for in the food eaten.

Barritt and King (3) likewise found that the variation in the cystine content of rabbit fur was due more to seasonal factors than to the quantity of cystine fed in the diet. Russell (31) found that when rations high or low in sulphur were fed no effect on the gross weight of the fleece, or on the percentage of clean wool, of sulphur in pure wool fibre, or of yolk, was apparent. Darlow *et al.* (11)

likewise failed to get significant responses from variations in amount of protein, or cystine, fed to wethers. Van Wyk *et al.* (36) obtained no increase in wool growth when a low protein ration was supplemented with either cystine, sulphates, potassium thiocyanate, or sulphur.

From the experiments reviewed above, one could conclude that the relation of dietary protein, cystine, or sulphur levels to wool growth has yet to be established. Further, the ability to produce normal wool probably is not lost during short periods of caloric or protein deficiencies when there is no impairment to health.

From our present knowledge of nutrition we believe that wool growth and quality may be indirectly affected by deficiencies of certain vitamins and minerals that are not associated with wool growth but with other bodily functions. Numerous experiments could be cited to show the need of various vitamins and minerals for the maintenance of growth, lactation, reproduction and health, but a survey of the literature has failed to reveal where wool growth was a factor in determining the role of any one vitamin or mineral. Pregnancy trials at the University of Alberta have proved that oat green feed is deficient in calcium. Numerous studies elsewhere have shown that there may be phosphorus or vitamin deficiencies in certain roughages. The prevalence of goitre has indicated deficiency of iodine. But in none of these trials was wool growth studied. However, there is reason to assume that unthriftiness caused by a mineral deficiency would be followed by decreased wool growth. Duerden *et al.* (14) secured a 32% decrease in fleece weight and a considerable decrease in body weight when they fed sheep on a phosphorus-deficient diet. Riggs (28) reported "improvement in growth, lustre and elasticity of the new wool grown after the administration of ferric ammonium citrate."

The purpose of summarizing the foregoing experiments has been to show how meagre and fragmentary is knowledge concerning the relation of wool growth and environment. It is generally accepted that a relation does exist. This is based on the differences in quantity and quality of wool produced by sheep maintained under different conditions of soil and climate over a long period of time. However, convincing evidence is lacking of the effect of a measurable difference in any one of the environmental factors. Some experimenters have attempted to measure such an effect. So far the results are either incomplete or contradictory.

To obtain the maximum growth and quality of wool, the environmental factors involved in its growth must be determined; then the sheep may be raised under optimum conditions. In many cases, the common methods of feeding and general management now in use are not conducive to maximum wool growth and quality. To shed more light on the problem of wool growth and to determine the influence of certain environmental factors on growth and quality of wool, the following trials were conducted.

### Outline of Experiment

In all of the five trials, nine lots of ewes (eight in each) were used. Each group was placed under the environmental condition indicated in the following list:—

- Lot 1. High temperature, high humidity, early shearing.
- 2. High temperature, low humidity, early shearing.
- 3. Low temperature, high humidity, early shearing.
- 4. Low temperature, high humidity, late shearing.
- 5. Low plane of nutrition.
- 6. Medium plane of nutrition.
- 7. High plane of nutrition.
- 8. Medium plane of nutrition, plus casein.
- 9. Medium plane of nutrition, plus minerals.

Lots 1 and 3 were the high and low temperature groups.

1 and 2 were the high and low humidity groups.

3 and 4 were the early and late sheared groups.

5, 6 and 7 were the low, medium, and high plane of nutrition groups.

8 and 6 were the high and low protein groups.

9 and 6 were the high and low mineral groups.

Significant differences in performance of the contrasted groups, therefore, would be a measure of the effect of the different environmental and nutritional conditions imposed.

### SHEEP USED AND METHOD OF ALLOTMENT

During the first three trials, grade and purebred ewes of different mutton breeds were used, while in the last two, ewes of Rambouillet breeding were used. This change was made to reduce the variability of the animals in any one lot.

In the first three trials (grade and purebred sheep), each comparable group had an equal number of ewes of each breed, as similar for age, weight, fleece weight, fleece length and quality as it was possible to obtain from the University flock of over two hundred. By this method of allotment it was assumed that there would be comparable pairs of individual animals in the respective lots. It was also assumed that by repeating the trials, sufficient data would be obtained to show significant differences in the performance of comparable groups.

However, owing to the variability of the results within the lots and because the results of the first year differed from those of the second in certain instances, it was deemed advisable to use animals that were more uniform in breeding, age, and wool characters. Range ewe lambs and yearling ewes were purchased in the fall of 1932 for the 4th and 5th trials. These ewes were from the same flock and were the result of several generations of Rambouillet breeding on the sire's side of the pedigree.

Although in all trials the allotment was made to secure equality of comparable lots, there was considerable variation among the individuals of any one lot. According to the Committee on Methods of Investigation for the American Society of Animal Production (1), "uniformity between lots is a *sine qua non* of experimental work. Uniformity within lots is desirable in the investigation of most problems, but is less important than that the lots be uniform when compared with each other." Dunlop (15) strongly criticizes the use of groups showing variation within themselves, as well as the group-feeding method used. He states, "no basis of allotment, at present available, predicts the relative or quantitative growth rate of animals with any degree of certainty" and refers the reader to numerous trials for proof. Snedecor and Culbertson (33) proved in a trial with pigs that an experimenter can reduce the probable error of the resulting mean differences by estimating the animals' performance or "outcome" and allotting them uniformly on this basis.

However, it was assumed that the method of allotment used in these trials would reduce the error of the mean differences below that resulting from random grouping of the experimental animals.

#### NUTRITION OF ANIMALS

Feeds commonly fed to sheep in Alberta were used throughout. The amounts of hay and grain (with the exception of Lots V and VII, the low and high plane of nutrition groups) were such as would carry the ewes through the trials in fair condition. The sheep consumed as much hay as desired, without undue waste. The amounts of grain ranged from 0.35 to 0.60 lb. per head daily, depending on the quality of the hay and the amount of grain necessary to enable the ewes to make normal gains during pregnancy.

Ground oats and upland prairie hay or prairie wool was fed in all trials. The hay varied in quality and composition from year to year and occasionally during a trial, but on the average it was of fair quality, at no time being exceptionally good or poor.

Owing to the scarcity or high price of prairie hay and the abundance of oat hay in Alberta, the latter was included in the rations for the 3rd, 4th and 5th trials. Oat hay (greenfeed) was fed every 3rd day from Jan. 2, 1932, to the end of the 3rd experiment. In the 4th experiment, oat hay was fed every other day throughout the trial. During the 5th experiment, lasting 12 months, prairie, oat, and western rye hays were fed.

The oat hay (greenfeed) was grown on the University farm and cut when the majority of the kernels had reached the dough stage. The leafiness and length of straw were also fairly uniform. The western rye hay was cut following blossoming and was quite well cured, making a satisfactory roughage for sheep.

While no chemical analysis of each hay was made, the average analysis of prairie and oat hay fed at the University of Alberta, and that shown in

"Feeds and Feeding" by Henry and Morrison, Table I, are as follows:—

—	No. of analyses	Moisture, %	Ash, %	Crude protein, %	Fibre, %	NFE,* %	Fat, %
Prairie hay	3 U. of A.	5.4	7.1	6.7	31.5	46.5	2.7
	42 H. and M.	6.5	7.7	8.0	30.5	44.7	2.6
Oat hay	5 U. of A.	10.0	6.2	9.6	33.9	47.4	2.9
	72 H. and M.	12.0	6.8	8.4	28.3	41.7	2.8

\* Nitrogen-free extract.

Technical casein formed the protein supplement to the diet of Lot VIII.

The "chemically pure" mineral supplements fed to Lot IX varied from year to year, and were as follows:

1st trial: calcium carbonate and sodium sulphate.

2nd trial: calcium carbonate and sodium sulphate.

3rd trial: calcium lactate and sodium sulphate.

4th trial: calcium lactate, mono sodium phosphate and sodium sulphate.

5th trial: calcium lactate, mono sodium phosphate and sodium sulphate.

#### LENGTH OF THE EXPERIMENTAL PERIODS

The length of the experimental periods for Lots I, II, and III were necessarily limited to the late fall and winter months, because both humidity and temperature differences between the comparable groups were directly dependent upon the difference between outdoor and indoor temperatures. During these months there is usually the greatest difference in the average outdoor and indoor (heated pen) temperature. The relative humidity of Lot II (low humidity pen) was dependent upon the number of degrees the outdoor air was raised to equal the desired indoor temperature. Therefore, in order to obtain the greatest differences in both humidity and temperature, the trials had to be limited to those winter months when average outdoor temperatures were low.

While it was not necessary to limit the experimental periods of the nutritional groups to the winter, it was believed that the conditions imposed on them were such as to cause significant effects on wool growth and quality during that period. The growth during the 90- to 100-day period would be one-third to one-quarter the length of each fibre that would be subjected to routine tests. Since, however, it could reasonably be expected that greater differences would develop between the wool of comparable groups by lengthening the experimental periods, this was done in the later trials, the last one continuing for 12 months.

#### Lots I to III

1st trial: Dec. 3, 1929 to Feb. 28, 1930; 87 days.

2nd trial: Dec. 2, 1930 to Mar. 10, 1931; 98 days.

3rd trial: Nov. 21, 1931 to Mar. 4, 1932; 104 days.

4th trial: Nov. 12, 1932 to Mar. 22, 1933; 130 days.

5th trial: Nov. 8, 1933 to Mar. 24, 1934; 136 days.

## Lots IV to IX

- 1st trial: Dec. 3, 1929 to Mar. 11, 1930; 98 days.
- 2nd trial: Dec. 2, 1930 to April 24, 1931; 143 days.
- 3rd trial: Nov. 21, 1931 to April 23, 1932; 154 days.
- 4th trial: Nov. 12, 1932 to June 2, 1933; 202 days.
- 5th trial: June 2, 1933 to June 7, 1934; 370 days.

The ewes in Lots V to IX of the 4th trial were left in the same lots throughout the 5th, except for several ewes in the nutrition groups that were re-allotted because of their condition. By continuing these lots into the 5th trial, it was hoped that the results would show an accumulative effect of the experimental conditions imposed.

The authors have failed to find published results of trials in which the effect of controlled temperature and humidity differences were studied. Most nutritional experiments, however, involve periods of six months or longer. Fraser and Nichols (19) compared carbohydrate- and protein-rich diets during a 98-day trial. Darlow *et al.* (11) reported their nutritional trials in which the experimental periods were from 108 to 180 days.

It is recognized that any experiment should be of sufficient duration to affect the performance of the animals in comparable groups. The greater the differences in the conditions imposed, *i.e.*, high *vs.* low protein, the shorter the time that should be necessary to produce an observable effect. It is also recognized that animals may store certain nutritional substances and be capable of performing normally for a longer or shorter period on deficient diets, depending on their reserve, their requirement, or degree of deficiency in one or more nutritional substances.

## METHOD OF SAMPLING FLEECES

To determine accurately the growth and quality of wool produced during the experimental period, samples of wool had to be taken at the beginning and end of each trial. Samples taken at the beginning represented normal wool grown since the previous shearing, during which time all experimental ewes received similar treatment. This first sample was removed from the middle of the right shoulder. At the end of the experiment, a similar sample was removed from the same area on the left shoulder, and represented wool grown prior to and during the experimental period. The difference between the two represented the wool grown during the experiment, and was a measure of the effect of the changed environment.

Experimenters elsewhere have employed different methods of sampling, none of which appeared suitable for the trials now being reported. Most trials covered yearly or half-yearly periods. When this was not the case, much larger numbers of sheep were used.

Fraser and Nichols (19), in their 98-day feeding trial, compared samples of wool that were removed from a tattooed area, prior to and after the trial, and expressed results as "a percentage change upon the conditions at the beginning of the experiment."

The criticisms that could be made of the method of sampling used in these trials are as follows: first, the shoulder sample is not representative of the fleece, and secondly, the wool grown on the right and left shoulders is not identical and therefore cannot be compared.

The mid-shoulder area was chosen for sampling because there was less matting and possibility of wear from rubbing on that part than on any other part of the body. Further, it did not appear necessary to take a sample that would be representative of the whole fleece because it was assumed that if the length, fineness, crimp, yolk, etc., of shoulder wool was affected by the experimental conditions, the wool grown elsewhere would be affected similarly.

At the time of planning the experiment, there was no published proof that wool grown on the right side was dissimilar to that grown on the left, and any cursory study that the authors have made of wool on the animal or of clipped fleeces has not shown obvious variation. Later work, however, has shown that there is a wide variation, even between locks adjacent to each other. According to Fairbanks (16), "when duplicate samples are taken one inch apart and from rather small areas, there are significant differences between the arithmetic mean of their diameters." However, granting the fact, Burns (5) reports that Roberts showed how a reduction from 25% to 5% variation can be secured by "quartering" to secure a composite sample for measurement. Thus, an average mean for fineness was secured that was exceeded by 5% not more than once in 250 times. These references are made to show the extent of the variations and, by the use of improved technique, how arithmetic means with a high degree of accuracy can be obtained.

One could expect, therefore, that by use of the proper technique, comparisons could be made of left and right shoulder samples. With this in mind, special shoulder samples were taken from the left and right sides of five of the experimental ewes and sent to the National Research Laboratories at Ottawa. Diameter measurements of fibres showed average differences as follows: 0.35%, 1.50%, 0.90%, 1.27%, and 0.31%, 120 to 125 fibres taken at random from each sample being measured. For tensile strength, differences of a little over 1% were found. Thus, for these wool characters very little difference existed between the left and right shoulder samples.

For staple and fibre length, differences up to 10% were found when 10 to 12 measurements were made. These might not have been as great had the number of measurements been increased. For grease, suint, dirt, and clean wool, the measurements of which were made in duplicate, the following differences were found between left and right shoulder samples:

—	Av. 5 samples, %	Range, %
Grease	2.27	0.63 to 3.79
Suint	7.04	0.00 to 14.49
Dirt	7.69	3.69 to 16.25
Clean wool	1.98	0.22 to 4.51

It is, therefore, apparent that for most wool measurements there was considerable similarity between left and right shoulder samples.

In experiments conducted for less than a year, a common method of comparison is to remove con-

secutive samples from the same area (6, 7, 18), or the animals are shorn twice yearly (11, 23). This method could have been used had not a number of ewes developed a bare spot in the area from which the first sample was removed. The clipped area was approximately 4 in. by 6 in., and the extremely cold weather which followed sampling may have been the cause of shedding. In some of the black-haired breeds, wool with a large mixture of black fibres grew at the edge of the clipped area. For these reasons it was impossible to take both samples from the same place.

The most satisfactory method of sampling depends upon the type of experiment as well as upon the desired data. It would appear from the literature and facts presented above that the method used was best suited to the trials reported here.

#### RECORDING OF THE DATA

All the ewes were weighed for three consecutive days at the beginning and end of the test period and the 3-day averages used as the initial and final weights. Additional weights were taken at the end of each 28-day period in order to follow the gains made by each animal. During the later trials, when the trial extended to and past the lambing period, pre- and post-lambing weights were also taken.

Weights of all feedings were recorded and the amount of hay refused or wasted was noted. Fleece weights were obtained at shearing time.

Breeding records of all ewes were kept, as well as details of their offspring at birth and subsequently.

Any abnormal conditions that developed during the trials, with possible bearing on the results, were noted.

Outdoor temperatures and humidities were observed twice or three times daily. The maximum and minimum temperatures were secured from records of the Field Crops Department. The outdoor humidity readings were taken as described later in this report.

Indoor temperatures and humidities of the heated pens were also recorded twice daily.

As previously mentioned, wool samples from the different groups were forwarded at regular intervals to the National Research Laboratories in Ottawa for critical analysis. For several years, entire fleeces from all the lots were subjected to special grading at the warehouse of the Canadian Co-operative Wool Growers' Association, Weston, Ontario.

The report of the "Laboratory methods used in the measurement of wool characters," being Part II of this series, appears in this issue.

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# THE INFLUENCE OF NUTRITIONAL AND CLIMATIC FACTORS ON WOOL GROWTH AND QUALITY

## II. LABORATORY METHODS USED IN THE MEASUREMENT OF WOOL CHARACTERS<sup>1</sup>

BY P. LAROSE<sup>2</sup> AND A. S. TWEEDIE<sup>3</sup>

### Abstract

The methods described were employed in the laboratories of the National Research Council for the examination of wool samples sent from the University of Alberta. The samples (as mentioned in Part I) were from sheep used to investigate the effect of various environmental factors on wool growth and quality.

The determination of crimp, staple length, fibre length, tensile strength, elongation, fibre diameter, moisture content, wool wax, suint, dirt, and yield of clean wool is described. The methods for the measurement of fibre diameter and for the determination of wool wax, suint, dirt, and clean wool involved new technique and some novel features; they are, therefore, described more fully than the others.

### Introduction

Wool clipped from the sheep described in Part I was sent to the National Research Laboratories for testing. There were 72 samples in each of the spring and fall clippings except in the fourth and fifth years, when some of the measurements were carried out on spring samples only.

The large number of samples to be tested made it imperative to use the simplest and most rapid methods consistent with the accuracy desired.

The following characters were measured and reported at one time or other during the experimental period: crimp, staple length, fibre length, tensile strength, elongation, fibre diameter, moisture content, wool wax, suint, dirt, clean wool.

Most of the determinations were carried out by one of the authors (A.S.T.). During the first year, the testing was under the direction of Mr. A. F. Gill, and the authors began work on the problem in the autumn of 1930.

### Methods

#### CONDITIONING

All physical tests were carried out on samples conditioned at 60% relative humidity and 70° F. except where fibre diameter was determined by the microprojection method, when it was necessary to remove the samples from the conditioning room for measurement. However, in this case, the humidity conditions during the measurement of comparable samples did not vary sufficiently to cause any appreciable change in diameter.

#### SAMPLING

Care was taken that the sample for the particular determination to be carried out was representative of the whole sample submitted for test.

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The general procedure was to divide the sample submitted into zones and pick out from each zone a number of locks. A small group of fibres was then taken from each lock and the groups combined. The small sample containing the required number of fibres for any one test was obtained from this composite sample by picking the fibres a few (4-6) at a time. This was the procedure followed in sampling for tensile strength and diameter determinations. For fibre length, the composite sample was omitted and the required number of fibres was picked at random directly from the representative locks. In the case of crimp and staple length determinations, the locks themselves were used. For the wax, suint, and dirt determinations, sampling was simplified by the fact that in the majority of cases the remainder of the original sample, after removal of portions for the other determinations, was utilized.

The number of fibres measured was sufficient in each case to give the desired degree of accuracy.

#### CRIMP

The crimp, as determined in the first year, was obtained by counting the number of waves or kinks in a given length of the individual fibres. The number of readings made on each sample varied between 40 and 60, the observations being made by two people on groups of 20 to 30 fibres. The readings were recorded to the nearest half crimp. The large number of readings was necessitated by the large variation found between individual fibres, e.g., 3-10, 1-7, 2-11, in any one sample. Because of these large variations and because the undulations were generally difficult to distinguish, the crimp in all subsequent samples was determined on the locks instead of on the individual fibres. A rule was held directly against the lock near the root end and the number of undulations in a known length, generally one inch, was counted. Measurements at ten different parts of the sample were usually sufficient to give the required accuracy. In a few cases the crimp was so irregular or ill-defined that the number of waves could not be counted accurately, and no record was made.

#### STAPLE LENGTH

The staple length was obtained by holding a rule directly against a lock of wool fibres and noting the distance from the clipped end to the tip. In the case of long wools where the tip of a lock tapered off, a mean length between the longer and shorter parts of the lock was taken as the staple length. The length was read to the nearest millimeter. As in the measurement of crimp, two sets of 20 to 30 observations were made on each sample during the first year. In the subsequent experiments it was found sufficient to carry out 10 measurements on each sample.

#### FIBRE LENGTH

The fibre length was determined by holding the ends of the fibre against a rule by means of forceps, using sufficient tension to remove the kinks without stretching the fibre. A convenient rule for the purpose was made by covering

one face of a wood slat with black paper, along one edge of which was glued a paper scale. The wool fibres were easily seen against the black background next to the scale.

When a Sever fibre length tester was acquired later, the method was checked with this apparatus and no significant difference was found in the results.

The lengths were read to the nearest millimeter, and two sets of 20 to 30 observations were made on each sample in the first year. In the second year, only 10 measurements were carried out on each sample, but in the subsequent experiments this number was increased to 20.

#### TENSILE STRENGTH

The tensile strength of the wool fibres was measured on a Mackenzie type of machine, described by Matthews (4) and by Hill (2).

The distance between the jaws of the machine varied for different samples according to their fibre length, but as far as could be ascertained by a few tests, varying the length within the limits reached in these experiments did not materially affect the results.

In the fourth and fifth years' experiments, instead of measuring the tensile strength on fall and on spring samples, separate measurements were carried out on the root and tip portions of the fibres, the same fibre being used for both determinations. The distance between the jaws was consequently short, being on the average about 2.5 cm. This change was made to decrease as much as possible the error resulting from the variation in strength of fibres and arising from the measurement of strength on two different sets of fibres. It was hoped in this way to render more evident the effect of the factor being investigated. Incidentally, this procedure also lessened the amount of work involved in carrying out the test, since only one sampling and cleaning of the wool was necessary instead of two.

The rate at which the load is applied is an important factor in the use of the Mackenzie machine. Generally, the more rapidly the force is applied to the fibre, the higher is the value obtained. In order to reduce this source of error, all tensile strengths were determined by one operator (A.S.T.) and at a low speed which was kept as constant as possible.

In all cases, the tensile strength was read to the nearest  $\frac{1}{2}$  decigram or within the limits 1 part in 100 to 1 part in 500, depending on the strength of the fibre. As a rule, two sets of 25 measurements were carried out for each sample but when the agreement between the two averages was not good, a third and sometimes a fourth set of 25 readings was taken.

#### ELONGATION

Elongation was determined at the same time as tensile strength, and therefore, the same remarks regarding number of observations and choice of samples apply here.

The Mackenzie tester has no automatic stop, and the attention of the operator must be continually directed to the elongation scale in order to read the elongation at the moment of rupture. Owing to the low sensitivity of

the scale and the large error possible in reading the elongation, the measurements were discontinued after the first two years.

#### FIBRE DIAMETER

Three different methods have been used in measuring the diameter of the wool fibres. In the first year's experiments, a number of fibres were placed in a roughly parallel fashion between a microscope slide and a cover glass, the fibres and cover glass being held in position by a little Canada balsam on opposite edges of the cover glass. The diameters were measured by means of a microscope fitted with a micrometer eyepiece. The micrometer was read to the nearest unit and since the magnification was such that one division of the micrometer screwhead corresponded to a cross hair movement of  $0.86 \mu$ , the accuracy of the readings was about 2%. Usually two sets of 25 measurements were carried out but when these did not agree within the required limits, additional sets were measured.

For the samples of the second year's experiment and the fall samples of the third year's experiment, the eriometer was used to determine the diameter of the fibres. The instrument was similar to that described by McNicholas and Curtis (5). Cards, having small circular openings of a size corresponding to that of the eriometer, were used to support the fibres. These were placed in parallel sets of ten over each opening.

Although convenient and rapid, the eriometer method was abandoned, for it was found that with fibres which varied widely in diameter in any one sample, consistent readings were difficult to obtain. Apparently, this was because the fibres generally divided themselves into two classes in the formation of diffraction bands. The larger fibres in a set seemed to group themselves in forming one set of diffraction bands while the finer fibres formed another, so that the readings depended on the position of the eye. This is the only fault that has been found with the eriometer and, no doubt, could be eliminated by sorting visually the finer from the coarser fibres of the sample, and then taking separate eriometer readings.

In making measurements with the eriometer, 5 groups of 10 fibres each were tested, 10 readings being taken on each group. These readings were made to the nearest  $0.3\text{--}0.4\mu$ , or with an accuracy of about 1 part in 100, for the fibres measured.

The remaining samples were measured by a micro-projection method making use of the apparatus described by Larose (3). Fibres from a sample were mounted on cards each holding about 60 fibres. The method of mounting the fibres on the cards is illustrated in Fig. 1, which shows the wooden blocks used when root and tip portions of the fibres were measured separately. This method was followed in the 4th and 5th years' experiments, for the reason given in the discussion of tensile strength. In the earlier experiments, where a single determination was made on each fibre, the central portion was usually measured. This gave rise to an error which will be discussed with the results.

The cards A and B were held by thumb tacks on the blocks C and D, which had two metal strips E and F, notched at  $\frac{1}{8}$ -in. intervals, to keep the fibres equidistant and parallel. When the fibres were measured at one point only, another block was used, which held apart the two metal strips at a distance equal to that of the width of a card. The two blocks illustrated were held apart at the correct distance, depending on the length of the fibres, by sliding them in or out on two nails G and H. On the outer edge of the blocks alongside the metal strips was placed a sticky wax J made of a

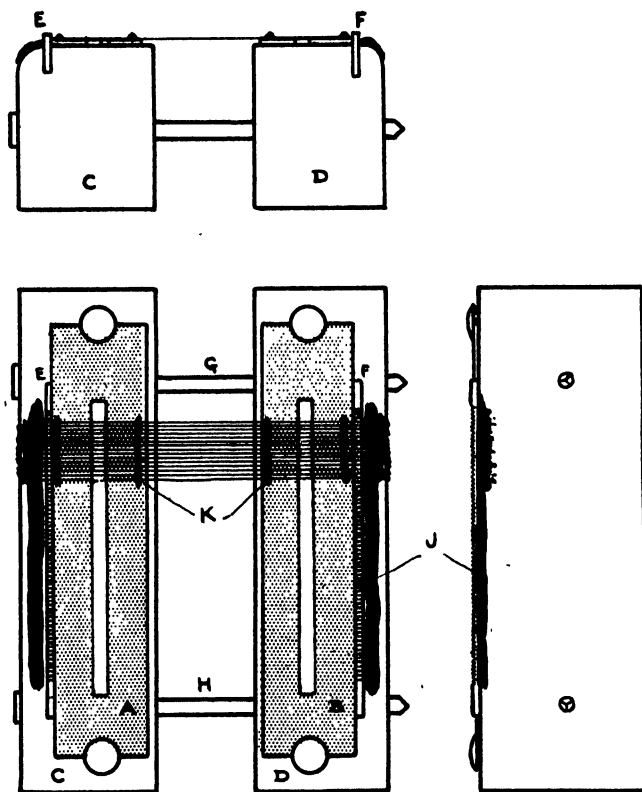


FIG. 1

mixture of beeswax, rosin, and wool wax, or vaseline. The consistency of this wax was such that a fibre could easily be pushed in by the finger, but once embedded it would not easily slip out. The cards were of a length equal to, and of a width about two-thirds that of a microscope slide. They had in the centre a longitudinal slit about 5 mm. wide and slightly longer than the traverse of the mechanical stage on the microscope, so that in moving the stage from one end to the other, the fibres could be brought successively in front of the objective. In mounting, one end of the fibre was fixed in the wax, the fibre passed through opposite notches in the metal strips, and the free end embedded in the wax on the other edge, with sufficient tension to

remove practically all crimp. When the required number of fibres had been mounted in the manner described, they were fixed to the cards on both sides of the slit by means of a hard wax K (rosin + paraffin + beeswax) applied in the molten state. When this wax solidified, the cards were removed and the free portions of the fibres cut away.

The microprojection apparatus gave a magnification of 1000 and the diameter of the image on the screen was measured to the nearest millimeter. This corresponded to an accuracy of  $1\mu$  in actual diameter or 1 part in 20 to 40 depending on the size of the fibre. Measurements were carried out usually on 120 fibres, but when the agreement between the two groups of 60 fibres each was not very good, a third and sometimes a fourth group of 60 fibres would be measured.

As some of the results obtained with the eriometer were to be compared with those obtained with the micrometer eyepiece, and others with results obtained with the microprojection apparatus, a comparison was made by carrying out measurements on a few samples by all three methods. The eriometer gave results which on the average were 1.5% higher than those obtained by the microprojection method, and 3.0% higher than those obtained by the micrometer eyepiece. However, a correction of these differences between methods was not essential, since the measurements made were always comparisons and any consistent error would, therefore, cancel out.

#### MOISTURE CONTENT

The moisture content of the wool as received was determined in the first two years' experiments only, for it was evident after these determinations that it had no special significance. In the second year the moisture was also determined after conditioning at 60% relative humidity.

The samples, weighing from 25 to 50 gm., were received in sealed tins. On opening the tin, the sample was divided into two portions which were weighed immediately. When the moisture content in the conditioned state was desired, the wool was conditioned at 60% relative humidity for at least a day, or until it reached the equilibrium state. In most cases it was found that more than one day was necessary. After weighing, the samples were dried at 105–110° C. for 3 hr. in a conditioning oven, and again weighed.

The moisture content was calculated on the dry weight to give the regain of the wool, as received and as conditioned at 60% relative humidity.

#### WOOL WAX, SUINT, DIRT, AND CLEAN WOOL

For the present purpose, wool wax is defined as that part of the raw wool which dissolves in petroleum ether, while suint is represented by the water-soluble portion. The dirt is any matter which does not dissolve in water or in petroleum ether, extraneous to the wool.

The method followed in determining the amount of wool wax, suint, dirt, and clean wool in the raw wool samples was as follows:

Duplicate samples of about 20 gm. of the wool (or the balance, if less than 20 gm. remained after the other determinations) were placed in extraction thimbles (43 × 123 mm.) which had previously been dried in an oven at

105° C. and weighed. The wool and thimbles were dried to constant weight at 105° C., 6 to 7 hr. generally being sufficient. The dry weight was determined without removing the samples from the oven by making use of a balance placed on top of the drying oven and with a suspension passing through the bottom of the balance and the top of the oven. All weights of dried material were obtained in this way so that cooling in a desiccator was not necessary and the change in weight on drying could easily be followed. Designating the weight of the thimble by A and that of the wool and thimble by B, the weight of the dry raw wool is given by  $B - A$ . The wool was then extracted with petroleum ether in a Soxhlet for 2 to 3 hr. Owing to the rapid distillation of the solvent and the frequent filling and emptying of the thimble, the extraction was found to be complete in this time, and was equivalent to one of longer duration with a higher boiling solvent such as benzene. The thimble and wool were removed and most of the adhering solvent allowed to evaporate in air, after which drying was carried out in the oven at 105° C., 2 to 3 hr. being sufficient in this case for constant weight to be reached. Designating this dry weight by C, the weight of matter extracted is  $B - C$ . The wool was then removed from the thimble and both were washed in three changes of warm distilled water, the washings being collected in a 2-litre beaker and allowed to stand overnight, during which time the dirt settled to the bottom. Washing in warm water removed most of the dirt. The coarse vegetable matter was removed by hand, leaving only a little fine vegetable matter of negligible weight adhering to the wool. The wool and the thimble were then dried at 105° C. to constant weight. Designating the weight of the dried wool by D and that of the thimble by E, the amount of suint plus dirt is given by  $C - (D + E)$ . The clear liquid in the beaker which had stood overnight was decanted and boiled down to a small volume (10–20 cc.). The residue was filtered on a Buchner funnel fitted to a suction flask and provided with a filter paper slightly larger than the funnel and previously weighed after drying at 105° C. The hot evaporated liquid was also passed through the filter which was then washed with small amounts of hot distilled water and finally dried at 105° C. and weighed. Designating this last weight by H and that of the filter paper by K,  $(H - K) + (E - A)$  represents the amount of non-water-soluble dirt, the second term being a correction for any dirt adhering to the thimble. In filtering the dirt from the wash liquors, the best technique found was to use a fairly strong vacuum on the suction flask at the start. Once the suint liquor had begun to filter through, a clamp on the connecting rubber tube was closed. If the filtration slowed up, the clamp was opened again for a short time.

The quantity of suint is determined by subtracting the amount of dirt found from that of suint and dirt previously determined. The amount of clean wool is, of course, given directly by D.

This method differs from that of Sutton (6) and the later modification described by Hill (1) in that petroleum ether is used as the wax solvent instead of ether as in the first, or benzene as in the second method, while the time of extraction is much shorter. The water treatment is also different,

because extraction with water has been found to have the following disadvantages. The thimbles are generally useless after one extraction. The method is long, and the apparatus has to be specially insulated to obtain the necessary boiling. The prolonged boiling-water treatment renders the wool unsuitable for further tests. It has also been found difficult to prevent dirt from siphoning over with the water. In a comparison of the two methods, it has been found that the simple washing with warm water gives results which are quite satisfactory. The determination of the wax and the suint by difference seems also to be more advantageous than the method of evaporating the respective solutions to dryness, as employed by Sutton and described by Hill. It has not been deemed necessary to carry out the alcohol extraction of any water-soluble matter removed with the wax, as this was found to be very small. The nature of this material seemed also to be different from that of the water-soluble suint, so it is questionable whether it should be classed as such.

The following figures show the results obtained with two wools in the comparison of the method described above (designated N.R.C. in the table) with that described by Hill. The figures are means of duplicate determinations and are calculated on the original weight of the dry raw wool.

Sample Method	698		699	
	N.R.C.	Hill	N.R.C.	Hill
Wax (by diff.)	13.5%	(14.0)%	10.5%	(10.9)%
Wax (by evap'n. -alc. ext.)		12.8		10.1
Suint (by diff.)	18.1	(18.0)	11.6	(12.1)
Suint (evap'n. +alc. ext.)		18.1		11.6
Dirt	4.0		5.0	
(by diff.)		5.0		6.0
(by diff. using bracketed fig.)		(3.9)		(4.7)
Clean wool	64.3	64.1	72.8	72.3

The figures given in brackets are the results of determinations not included in Hill's method. The slight deviation of the totals from 100% is due to omission of the second decimal.

The smaller amounts of wax obtained by the evaporation method are probably due to decomposition accompanying drying. For this reason, the determination of these constituents by difference was considered preferable. For the same reason, the amount of dirt determined by difference is likely to be too high.

Part III of this series will deal with the results of these experiments and a general discussion of their significance.

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## AN ANNOTATED LIST OF THE DIPTERA (FLIES) OF ALBERTA<sup>1</sup>

BY E. H. STRICKLAND<sup>2</sup>

### Importance of Flies

The Order Diptera is one which does not make a very great appeal to the average collector of insects. Vast numbers of species are very similar in appearance and few of those which occur in Canada are sufficiently striking in color or in appearance to attract the interest of an amateur.

For this reason the regional distribution of the flies which inhabit this Dominion is very imperfectly known. This is a serious handicap in connection with many problems of great importance to agriculture and to the health of man and animals. Members of the Order Diptera have a more varied influence upon human welfare than do those of any other Order of insects. Comparatively few feed directly upon living vegetation, though among these are included such serious pests as leather jackets, Hessian flies, fruit flies, and root maggots. On the other hand, to this Order belong many species of the utmost value to the field husbandman. Nearly all of the species in certain families live as parasites or predators on plant-feeding insects. To the family Tachinidae, for instance, must be attributed most of our immunity from severe and continued losses from many species of injurious caterpillars. Other species of flies constitute the most effective biological factor in the control of grasshopper populations. Still others play a most important part in reducing the numbers of plant lice. In addition, many prey upon their blood-sucking relatives and help to hold their numbers in check although, it must be admitted, not always with complete success.

Human interest in the manifold activities of flies undoubtedly centres around those species which, at some stage in their development, feed on the blood of man and livestock. Not only do they cause serious annoyance and pain by their methods of extracting blood, but many of them are also capable of transmitting fatal diseases while so doing. One has but to recall such human fly-borne diseases as malaria, sleeping sickness, or yellow fever to realize the part that disease-transmitting flies have played in the destiny of nations. To these must be added the contaminative diseases spread by houseflies and their associates.

<sup>1</sup> *Manuscript received January 18, 1938.*

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Finally, we should mention the direct attacks which are made by the larvae of certain flies on the flesh of man and livestock. Myiasis, the infestation of wounds by fly larvae, is becoming increasingly frequent among inhabitants of Alberta, while bot flies of horses, cattle, and sheep are a constant drain on the profits of animal husbandmen.

Tribute must, however, be paid to those fly larvae which have been brought into our province occasionally, in order that they may assist in the cure of bone and other human diseases. All of the species which have been thus employed belong to the native fauna of Alberta. The demand for their employment is, fortunately, not sufficient to warrant the expense entailed in raising "surgical maggots" locally.

### **The University of Alberta Insect Collection**

During the past fifteen years an attempt has been made, at this University, to compile a complete list of all species of insects which have been recorded as occurring within the confines of the province.

With this object in view, extensive collections have been made annually, and records of additional captures have been obtained from other sources, particularly from the Dominion Entomological Laboratory at Lethbridge.

Many specialists, both in Canada and the United States, have generously examined and named material in the University collection. Without their help the compilation of this list would not have been possible; we take this opportunity to thank them for their invaluable assistance.

In addition to extensive collecting, all available literature has been searched for published records of insects which have been captured in Alberta. Such Canadian publications as the *Canadian Entomologist* and the *Entomological Record* naturally have yielded a large number of records, but many which have never appeared in Canadian literature have been found in journals published in the United States.

### **Species of Diptera Captured in Alberta**

The card catalogue of the University of Alberta insect collection shows that nearly fourteen hundred species of flies have been authoritatively determined as belonging to the Albertan fauna. In addition to these, we possess series in several Families in which we have been unable to have determinations made by any specialist. In a number of instances we have been able to name many of the species ourselves, with the aid of the literature and of material from elsewhere which had been classified by specialists. We feel confident that the collection already contains two or three hundred additional species which cannot be named, under existing conditions.

As we have inferred, there are no amateur collectors of Diptera in Alberta. Apart from accounts in the literature, we have practically no records other than those of captures made by people who are associated with this University. As yet, no attempts have been made to collect flies in the less accessible

areas in the northern part of the province. The number of species which are represented by one or two specimens only is an indication that more intensive and extensive collecting in this province should greatly increase the record of captures which are listed here.

We believe, however, that the list is more complete than that of the other prairie provinces would be; it has been compiled as an initial contribution to the very imperfectly known Dipterous fauna of this part of Canada.

### Ecological Areas in Alberta

In recording localities of captures, it is the usual custom to give the name of a town or village in the vicinity of which the insects were taken. Many of the villages near which collections have been made are so small that residents in other parts of the province may be unaware of their location, or of their existence. Such records, therefore, are of very little value. The province can, however, be divided into a number of moderately well-defined ecological areas.

The southeast portion lies in the Transition Zone of the Austral Region. It is typically a short-grass "prairie" country, adapted to the production of hard spring wheat.

At the extreme southeast of this Zone there is an intrusion of the Cypress Hills, in which the flora and fauna closely resemble those of the Rocky Mountains in the southwest of the province. They differ considerably from those of the mountainous regions further north.

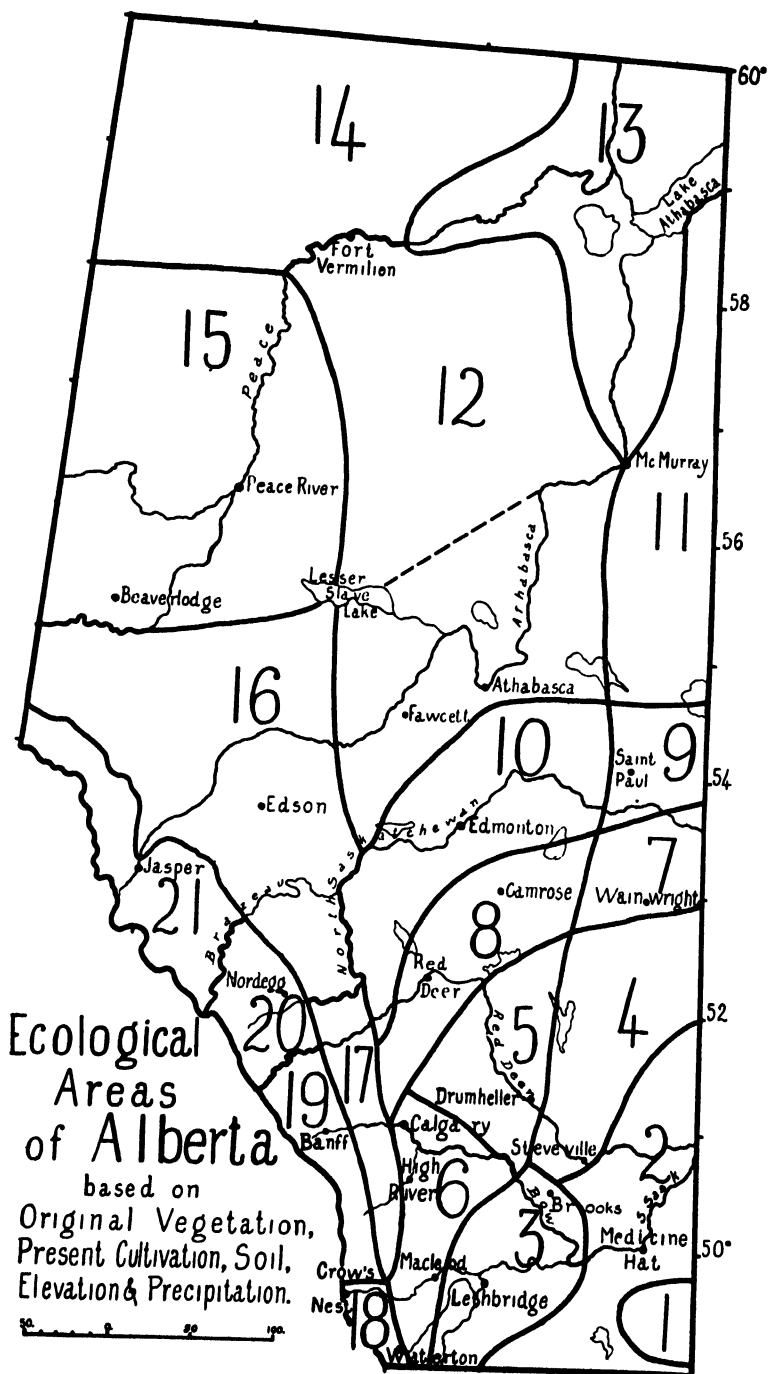
The remainder of the province lies in the Boreal Region and is almost entirely in the Canadian Zone, though it merges into a Mountainous Zone in the west through a foothill area, and into the Hudsonian Zone in the extreme north.

Variations in precipitation further divide the province into a series of dry areas in the east, as opposed to more humid western areas.

The soil is moderately uniform in the southeastern areas (Transition Zone); elsewhere it is so variable that any attempt to subdivide areas of prevailing vegetation and precipitation, on the basis of soil types, would necessitate the definition of over one hundred such areas. The flora is similar throughout each of the large northern areas outlined on the map (Fig. 1), though naturally it varies locally on the smaller areas of diverse soil. This variation is not as great as might be anticipated, since with the exception of part of area 15 comprising the "Peace River District", all northern soils are grouped into what are known as "Gray wooded soils". Throughout this vast territory nearly all the soluble chemicals have been leached out of the soil, whatever its physical texture may be.

Insect species which have been taken only in a particular locality in these areas are recorded as "12 sandy", "15 muskeg", etc.

The areas, as outlined, have been established with the assistance of Dr. E. H. Moss, of the Department of Botany, and of Dr. F. A. Wyatt, of the



**FIG. 1**

Department of Soils. The compiler has had to introduce a number of compromises between such areas as would be defined entirely on botanical considerations on the one hand, and on edaphic considerations on the other.

In computing rainfall, the "Long Time Average Annual Precipitation" chart published by the Searle Grain Co., has been utilized. Any precipitation (chiefly snowfall) between Nov. 1 and March 31 is not included in the chart. Most of this constitutes "run-off" and has little biological significance, except in so far as it may increase the breeding areas for aquatic larvae in the spring, and protect the underlying soil from intense freezing during the winter. Generally speaking, areas in the Transition Zone are bare for the greater part of the winter, whereas elsewhere the ground is covered with a foot or more of snow from early in November till the middle of March.

A brief description of each of the 21 selected areas is as follows:—

#### TRANSITION ZONE

##### 1. *Cypress Hills*

Vegetation:— About 50% forested; lodgepole pine, spruce, aspen and willow. Remainder; long grass. Very little cultivation.

Elevation:— Up to 4,500 ft.

Soil:— Very dark brown. Summit of hills never glaciated.

Rainfall:— 10–11.5 in.

Remarks:— Flora and fauna are very similar to those of Waterton Area, No. 18. No intensive insect collections have been made here.

##### 2. *Southern Prairie (Dry) (Medicine Hat)*

Vegetation:— Short grass. A few poplars in river bottoms. Cactus and sage in driest localities. Crops: chiefly grain. Much deserted land, which has grown up to mustard or Russian thistle.

Soil:— Fine brown clay, inclined to be sandy in eastern half.

Rainfall:— Less than 10 in.

Remarks:— Fauna near Medicine Hat includes scorpions, solpugids, white ants, horned toads and rattlesnakes. Nowhere else, east of the Rocky Mountains, do these forms extend into Canada. Little insect collecting in this area except in the vicinity of Medicine Hat.

##### 3. *Southern Prairie (About 50% Irrigated) (Lethbridge)*

Resembles Area No. 2. Vegetation on dry areas similar, irrigated parts carry a greater variety of crops, among which alfalfa and beets predominate. Soil a little heavier.

Remarks:— Extensive collections of insects have been made in this area, particularly around Lethbridge.

TRANSITION ZONE—*concluded*.4. *Northern Prairie (East) (Vermilion)*

Vegetation:— Short to moderately long grass. Much deserted land.  
Crops: almost entirely grain.

Soil:— Dark brown loam.

Rainfall:— Less than 10 in. Very light in eastern half.

Remarks:— Little insect collecting. The "badlands" of the Red Deer River are in the southwest corner of this area, near Steveville; but no collections have been made here to date.

5. *Northern Prairie (West) (Drumheller)*

Vegetation:— Moderately long grass. Crops: grain.

Soil:— Heavy clay "gumbo" to dark brown loam.

Rainfall:— 10–11.5 in.

Remarks:— Very little insect collecting except near Drumheller.

6. *Northern Prairie (Southern Extension) (Calgary)*

Vegetation:— Moderately long grass, occasional groves of willow and aspen. About 50% under cultivation. Crops: grain and hay.

Soil:— Dark brown loam.

Rainfall:— 10–11.5 in.

Remarks:— Spasmodic insect collecting has been conducted around Calgary, little elsewhere.

## INTERMEDIATE BETWEEN TRANSITION AND CANADIAN ZONES

7. *Parkland (East) (Wainwright)*

Vegetation:— About 30% wooded; aspen and willow groves. Remainder; moderately long to short grass. Crops: grain.

Soil:— Dark brown loam, with large areas of almost pure sand.

Rainfall:— Less than 10 in.

Remarks:— Few insects have been collected here; chiefly in the sand-dune country.

8. *Parkland (West) (Red Deer)*

Vegetation:— Originally about 50% wooded; mainly aspen. Remainder; long grass. Now about 70% cleared. Crops: grain and hay.

Soil:— Dark brown loam.

Rainfall:— 10–13 in.

Remarks:— Scattered insect collections have been made throughout this area, most intensively a few miles west of Red Deer.

## CANADIAN ZONE

9. *Poplar (East) (Saint Paul)*

Vegetation:— Originally balsam poplar, aspen, and willow, with some spruce. Less than 50% cleared. Crops: chiefly oats.

Soil:— Black loam.

Rainfall:— About 10 in.

Remarks:— Practically no insect collections have been made here.

10. *Poplar (West) (Edmonton)*

Vegetation, as No. 9, but with considerable stands of spruce and pine locally. About 70% cleared. Crops: largely wheat and oats, some hay.

Soil:— Black loam, with a high humus content.

Rainfall:— Over 13 in.

Remarks:— Insects have been collected intensively in this area, particularly in the vicinity of Edmonton and around several lakes further west.

11. *Mixed Forest with Eastern and Sub-arctic Intrusions*

Vegetation:— Aspen, balsam poplar, spruce, jack pine, balsam fir, tamarack, willow, birch, and alder.

Soil:— Gray wooded, with extensive areas of sand.

Rainfall:— Probably 10–11.5 in. No complete records.

Remarks:— This area is not well known. No insect collections have been made here.

12. *Mixed Forest, with Cordilleran (Rocky Mountain) Intrusions (Fawcett)*

Vegetation:— Chiefly forested with aspen, balsam, poplar, spruce, pine, etc. Numerous lakes and large areas of muskeg. A little cultivation in the south. Crops: chiefly oats.

Soil:— Gray wooded, very variable in texture.

Rainfall:— 11.5 to over 13 in.

Remarks:— In the present state of our knowledge there appear to be no factors on which this large area can be subdivided. On the map we have inserted a broken line to divide it, provisionally, into a northern and a southern "sub-area".

Insects have been collected, in rather small numbers, at a few places to the south and east of Lesser Slave Lake, particularly around Fawcett. None have been taken in the northern sub-area.

## CANADIAN ZONE—concluded.

13. *Mackenzie Lowlands*

Vegetation:— Mixed forest, as in No. 11, with more numerous Alpine-arctic species. Long grass and sedges in open spaces. No cultivated land.

Soil:— Incompletely surveyed, all gray wooded.

Rainfall:— No record, probably around 13 in.

Remarks:— Only about a dozen Dipterous insects have been received from this area.

14. *Mixed Forest with Some Parkland, and Alpine-arctic Intrusions*

Vegetation:— Similar to Nos. 12 and 15. No cultivation.

Soil:— Incompletely surveyed, all gray wooded.

Remarks:— This country is not well known. No insect collections have been made here.

15. *Mixed Forest and Parkland (Beaverlodge)*

Vegetation:— Large mixed forest areas interspersed with long-grass open plains. Crops: chiefly grain and hay.

Soil:— About 10–15% black loam; remainder gray wooded, with scattered patches of sand.

Rainfall:— 10–13 in.

Remarks:— Insects have been collected moderately intensively in the cultivated parts of the Peace River District.

## FOOTHILL ZONE

16. *Foothills (Northern)*

Vegetation:— This is an extensive area in which the flora is intermediate between that of the northern Rocky Mountains and the mixed forest of northern Alberta. Much of this area is incompletely surveyed. In a small portion of the eastern half some grain and hay are produced.

Soil:— Gray wooded. Believed to be very variable.

Rainfall:— Over 13 in.

Remarks:— No insect collections have been made in this area.

17. *Foothills (Southern)*

Vegetation:— Aspen, spruce, lodgepole pine, willow, with open prairie southward.

Soil:— Gray wooded in northern portion, dark brown to black in south.

Rainfall:— 13 in. in northern part to less than 10 in. in south.

Remarks:— This is possibly the least uniform area of those selected, even though it is one of the smallest. Very few insects have been collected here.

## MOUNTAIN ZONE

The four areas into which this zone has been divided each includes a district in which moderately intensive insect collections have been made.

**Vegetation:**— This naturally varies greatly with the altitude of the montane, submontane, subalpine and alpine territory, also with topography, rock and soil. Montane territory is dominated by lodgepole pine and white spruce. Douglas fir is locally abundant. Subalpine territory is characterized by Engelmann spruce, alpine fir, and other conifers, as well as by mountain heaths.

From Area 18 to Area 21 there is a gradual and irregular replacement of southern and western species by certain boreal and arctic species. The same characteristics appear to apply to the insect fauna.

### 18. *Southern Rocky Mountain (Waterton and Crow's Nest Pass)*

**Vegetation:**— Strong intrusions of southern and western species. These extend to about the northern limits of this area.

**Soil:**— Contains a relatively higher percentage of lime than does that of the more northern mountain areas.

**Rainfall:**— Over 13 in.

**Remarks:**— Intensive collections have been made in Waterton Park, at the extreme south of this area. Smaller collections from Crow's Nest Pass appear to be similar in composition.

### 19. *Central Rocky Mountain (Banff)*

**Vegetation:**— Typical for that of the Mountain Zone described above, with few southern or arctic intrusions.

**Soil:**— Very variable in texture, all gray wooded.

**Rainfall:**— Over 13 in.

**Remarks:**— Insect collections have been made at Banff, and to a lesser extent at Lake Louise. Elsewhere the fauna is practically unknown.

### 20. *North Central Rocky Mountain (Nordegg)*

**Vegetation:**— Similar to that of No. 19. Among the grasses are several species which appear to be rather typical of the Labrador flora. Mr. Kenneth Bowman, who has collected Lepidoptera in this area for many years, has taken a number of species in this Order which also were formerly considered to be peculiar to the Labrador fauna also.

**Soil and Rainfall:**— As in No. 19.

**Remarks:**— Small collections of Diptera have been made in this area in the immediate vicinity of Nordegg.

**MOUNTAIN ZONE—concluded.****21. Northern Rocky Mountain (Jasper)**

**Vegetation:**— As in Nos. 19 and 20, but with strong intrusions of arctic and boreal species.

**Soil and Rainfall:**— As in No. 19.

**Remarks:**— Little insect collecting has been accomplished at Jasper to date. Practically no flies have been received from here.

Collections of Diptera have not been made at a distance of more than about 50 miles from the town named (in brackets) in the above headings. Occasional specimens, received from elsewhere in these areas, have invariably belonged to species which have been taken in the neighbourhood of these towns. Should it be desired to make reference to a definite locality, in referring to any particular capture, the name of the town can safely be employed as an alternative to the number of the Area in which it is located.

**Explanation of Terms Employed in This List**

All species which are represented in the University of Alberta collection are marked with an asterisk (\*). Following the name of each such species are given the initials of the authority who made the determination.

The full names to which these initials refer are as follows:—

- J.A. The late J. M. Aldrich, U.S. National Museum, Washington.
- C.A. C. P. Alexander, Massachusetts Agricultural College, Amherst.
- J.B. J. C. Bequaert, School of Tropical Medicine, Harvard University.
- F.C. F. R. Cole, Redlands, California.
- C.C. C. H. Curran, American Museum of Natural History, New York.
- C.F. C. L. Fluke, University of Wisconsin, Madison, Wis.
- D.H. D. E. Hardy, Brigham Young University, Utah.
- E.H. The late E. Hearle, Dominion Entom. Laboratory, Kamloops, B.C.
- H.H. H. C. Hockett, Long Island Research Farm, New York.
- C.J. The late C. W. Johnson, Boston Natural History Museum, Boston.
- F.M. F. O. Morrison, University of Alberta, Edmonton.
- R.M. R. B. Miller, University of Alberta, Edmonton.
- C.S. C. W. Sabrosky, East Lansing, Michigan.
- R.S. R. C. Shannon, Cornell University, Ithaca, New York.
- F.S. F. M. Snyder, Minnesota State College, St. Paul, Minn.
- A.S. A. Stone, Bureau of Entomology, Washington.
- E.S. E. H. Strickland, University of Alberta, Edmonton.
- G.S. G. E. Shewell, Entomological Branch, Ottawa.
- C.T. C. R. Twinn, Entomological Branch, Ottawa.
- G.W. G. S. Wallev. Entomological Branch. Ottawa.

No data regarding the authority who made the determination are given with records obtained from the literature, or with those from other sources if the species is not represented in the collection. These are often unknown and the inclusion of the literature references would increase the size of this publication without a corresponding advantage. A few of the records from Lethbridge (Area 3) and from Waterton (Area 18) have been obtained from Mr. H. L. Seamans of the Dominion Entomological Laboratory at Lethbridge, and have not appeared previously in print.

No attempt has been made to give the names of collectors. Nearly all of the species recorded from the University collection have been taken by the compiler. Other collectors who have donated material to this collection are Owen Bryant, the late F. S. Carr (Medicine Hat, Area 2), the late Eric Hearle (Mosquitoes from Banff, Area 19), and a few university students.

Arabic numerals which follow each insect name refer to the Ecological Areas in which the species is known to occur. Roman numerals refer to the months in which the *adults* have been taken.

Thus:— “\**Tabanus astutus* O.S. (A.S.) 12, 20, 21. VII–VIII.” is interpreted as follows:—

“*Tabanus astutus* O.S. is represented in the University collection, classified by Dr. A. Stone of Washington, D.C. Adults have been taken in Ecological areas 12, 20 and 21, during July and August.”

Further, it can be deduced from the map that this species occurs in Lesser Slave Lake, Nordegg, and Jasper districts.

“\**Epistrophe sexpunctatus* Walk. ? (C.C.) . . .” indicates that Dr. Curran doubtfully referred material in our collection to this species.

“\**Helina obscurata* Mg. (H.H.) (= *nasoni*) . . .” indicates that the species has been recorded under both names as occurring in Alberta. In the majority of instances this indicates synonymy, but in some cases it refers to a misidentification of the species on the part of one writer.

#### PTYCHOPTERIDAE. False Crane flies

- \* *Ptychoptera* *metallica* Walk. (C.A.) 10. VII.

On two occasions we have received larvae, probably of this species, which were numerous in spring water piped into buildings.

#### TRICHOCERIDAE. Winter Crane flies

- Trichocera* *maculipennis* Fab. 10. V.

#### TIPULIDAE. Crane flies

The majority of the species recorded are the result of a very intensive collection made by Mr. Owen Bryant in 1924. These have already been published by Dr. C. P. Alexander in the Canadian Entomologist, Vol. 59, 1927. The comparatively small University collection contains only a few additional records in this Family. Dr. Alexander has, very kindly, examined this collection and has sent us a complete record of Tipulidae which are known to occur in Alberta.

TIPULIDAE—*continued*.

## TIPULINAE

- Prionocera** fuscipennis Lw. 10, 19. V & VII.
- \* sordida Lw. (C.A.) 10, 12. VI.
  - \* dimidiata Lw. (C.A.) 8, 10. VI-VIII.
  - \* **Nephrotoma** altissima O.S. (C.A.) 2, 3, 5-10, 15, 20. V-VIII.
  - \* erythrophrys Will. (C.A.) 2, 4, 7, 8, 10, 15, 20. V-VII.
  - \* ferruginea Fab. (C.A.) 2, 3, 8, 10. VI-VIII.
  - pedunculata Lw. 10. VII.
  - occipitalis Lw. 10. VI-VII.
  - Tipula** pachyrhinoides Alex. 12. VIII.
  - accurata Alex. 18, 19. VII-VIII.
  - barbata Doane 10. IX.
  - subbarbata Alex. "Alberta".
  - bucera Alex. 19. VIII.
  - \* mainensis Alex. (C.A.) 10, 12. VIII.
  - triplex Walk. (= umbrosa) 12. VIII.
  - penobscot Alex. 10. VII.
  - entomophthorae Alex. 10. VI-VII.
  - variata Alex. 10. VI-VII.
  - albertensis Alex. 10. VII.
  - latipennis Lw. 10. VII.
  - ingrata Dietz. 10. VII.
  - appendiculata Lw. 10. VII.
  - athabasca Alex. 10. VII.
  - \* macrolabis Lw. (C.A.) 10, 20. VII.
  - \* youngi Alex. (C.A.) 8, 10. V-VII.
  - balioptera Lw. 10. VI-VII.
  - fultonensis Alex. (= hinei) 10, 12. VI.
  - canadensis Lw. 10. VI-VII.
  - \* dorsimaculata Walk. (C.A.) (= angustipennis) 2, 3, 6, 10, 19. V-VII.
  - \* sarta Lw. (C.A.) 4, 7, 10, 12. V-VI.
  - senega Alex. 10. V-VI.
  - fragilis Lw. 12. VIII.
  - commiscibilis Doane 6. VII.
  - kennicotti Alex. (= parvemarginata) 6, 10, 12. V-VIII.
  - sulphurea Doane 10, 19. V-VII.
  - pendulifera Alex. 12. VIII.
  - grata Lw. 10. VII.
  - platymera Walk. 19.
  - tesellata Lw. 19.

The two preceding records are from the Entomological Record, 1913. They are not included in Alexander's list.

## CYLINDROTOMINAE

- \* **Lioigma** nodicornis O.S. (C.A.) 10. VI-VII.

## LIMONIINAE

- Limonia** triocellata O.S. 10, 12. VIII.
- sciophila O.S. 19. 5,700 ft. VII.
  - cinctipes Say 10. VI-VII.
  - solitaria O.S. 10, muskeg. VI-VIII.
  - dietziana Alex. (= gracilis) 10, VI.
  - tristigma O.S. 12. VIII.

TIPULIDAE—*continued*.LIMONIINAE—*concluded*.**Limonia**—*concluded*.**(Rhipidia)** *maculata* Meig. 10, 12. VI-IX.*fidelis* O.S. 10. VI-VII.**(Discobola)** *annulata* L. (= *argus*) 12. VIII.**(Dicranomyia)** *immodesta* O.S. 10, 12. VIII and X.*rostrifera* O.S. 12. VIII.*decora* Staeg. 12. VIII.*nycteris* Alex. 10. VII.*rufiventris neomorio* Alex. 10. VII.*halterata* O.S. 10. VI-VII.*athabasca* Alex. 10. VI.*haeretica* O.S. 10. VII.*intricata* Alex. 12. VIII.*platyrostra* Alex. 12. VIII.*melleicauda* Alex. (C.A.) 10. IX.**Helius** *flavipes* Macq. 10. VI.**Elliptera** *astigmatica* Alex. 12. VII.**Dicranoptycha** *quadrivittata* Alex. 19. VII.

## PEDICINI

**Ornithodes** *harrimani* Coq. 19. 5,700 ft. VII.**Pedicia** (*Tricyphona*) *rubiginosa* Alex. 19. 6,000-7,000 ft. VII.*constans* Doane 12. VIII.**Dicranota** *montana* Alex. 19. VII.**Ula** *elegans* O.S. 10. VI-VII.

## HEXATOMINI

**Epiphragma** *fascipennis* Say 10. VI.\* **Pseudolimnophila** *noveboracensis* Alex. (C.A.) 10, 12. VI-VIII.\* *inornata* O.S. (C.A.) 10, 12. VI.**Phyllolabis** *lagganensis* Alex. 19. VII.**Limnophila** *poetica* O.S. 10, 20. VI-VII.*bryanti* Alex. 10. VI.*harperi* Alex. 10. VI.**(Phylidorea)** *platyphallus* Alex. 10. VI.*tepida* Alex. 19. V-VI.*fuscovenosa* Alex. 19. VII.**(Ephelia)** *aldrichi* Alex. 19. VII.*medunnoughi* Alex. 19, 20. VII.\* **Pifaria** *recondita* O.S. (C.A.) 10. VI-VIII.**Hexatoma** (*Eriocera*) *alberta* Alex. 3. VI.

## ERIOPTERINI

**Neolimnophila** *ultima* O.S. 10, 12, 19. VIII-IX.**Gonomyia** *filicauda* Alex. 19. IX.**(Idiocera)** *icasta* Alex. 19. VI.**Gnophomyia** *cockerelli* Alex. 3. VI.**Rhabdomastix** (*Sacandaga*) *subcaudata* Alex. 19. VIII.*subfasciger* Alex. 19. VII-VIII.\* **Chionea** *valga* Harr. (C.A.) 10. XI-XII.

This wingless Tipulid is occasionally moderately abundant on the surface of snow around Edmonton.

## TIPULIDAE—concluded.

## ERIOPTERINI—concluded.

**Chionea** nivicola Doane 19. III.

This record is from the Entomological Record, 1910. It is not included in Alexander's list.

\* **Helobia** hybrida Meig. (C.A.) 2, 10, 12, 19, 20. IV-X.

**Ormosia** arcuata Doane 12. VIII.

albertensis Alex. 19. VII.

garretti Alex. 19. VII.

\* **Erioptera** villosa O.S. (C.A.) 10. VII.

bryantiana Alex. 10.

\* (**Psiloonopa**) manitobensis Alex. (C.A.) 12. VI.

**Molophilus** soror Alex. 12. VIII.

## ANISOPIDAE.

**Anisopus** punctatus Fab. 6.

## BLEPHARICERIDAE. Net-winged midges

**Philorus** aylmeri Garr. 19. VIII.

**Bibiocephala** griseus Curr. 20, 21. VI.

## SIMULIDAE. Black flies

**Simulium** arcticum Mall. 19. VII.

decorum Walk. 8. VIII.

hunteri Mall. 19, 21. VIII.

\* **pictipes** Hag. (C.C.) 3. VII.

\* **venustum** Say (C.C.) 2, 10, 15, 18, 21. VI-VIII.

vittatum Zett. "Alberta". VIII.

**Prosimulium** fulvum Coq. 2, 6, 19. VIII.

pancerastes D. & S. 19. VIII.

## CHIRONOMIDAE. Midges

The study of this family has been seriously neglected in Alberta, largely due to the difficulty in preserving specimens during general collecting in all Orders. The following records, therefore, constitute a small percentage of the species which occur here.

\* **Crictopus** brunnicans Walley (R.M.) 10. V.

\* **flavibasis** Mall. (R.M.) 10. Bred.

\* **tremulus** L. (R.M.) 10. V.

\* **Tanypus** monilis L. (C.C.) 3. VII.

\* **illinoensis** Mall. (R.M.) 10. VIII.

\* **Tanytarsus** fatigans Joh.? (R.M.) 8, 10. VI-VIII.

\* **Chironomus** barbipes Staeg. (C.C.) 3, 8, 10. V-VIII.

\* **brachialis** Coq. (R.M.) 10. VI-VII.

\* **dimorphus** Mall. (R.M.) 8. VI.

**farinalis** Walley 3. VI-VIII.

\* **imperator** Walley (C.C.) 3, 10. VII-VIII.

\* **indistinctus** Mall. (R.M.) 8, 10. V-VI.

\* **modestus** Say (C.C.) 3. VII.

\* **nigricans** Joh. (R.M.) 8, 10. VI-VIII.

\* **plumosus** L. (R.M.) 6, 10. V-VIII.

\* **tentans** Fab. ? (C.C.) 3. V.

## CHIRONOMIDAE—concluded.

- \* **Chironomus** utahensis Mall. (R.M.) 10. V.
- \* varipennis Coq. (R.M.) 10. VI.
- \* viridicollis V.d.W. (R.M.) 10. VII.
- \* viridis Mcq. (R.M.) 10. V and VIII.

## CERATOPOGONIDAE. Biting midges

- \* **Forcipomyia** cilipes Coq. (G.W.) 3, 10. VII-IX.

This non-biting species has been bred from muskrat dung in large numbers.  
The adults enter houses freely in late summer.

**Ceratopogon** cockerelli Coq. 19. VIII.

## PSYCHODIDAE. Moth flies

- Psychoda** prudens Curr. 20. VII.
- \* severini Tonn. (C.C.) 10. X.
- Pericoma** alberta Curr. 18. VII.

## DIXIDAE.

- Dixa** centralis Lw. 19. VI.
- johannseni Garr. 19.
- occidentalis Garr. 19.

## CULICIDAE. Mosquitoes

## CORETHRINAE Non-biting mosquitoes

- \* **Chaoborus** crystallina deG. (C.T.) 8, 10, 15. VI-VII.

The larvae of this species, which are predatory on those of biting species, are common in pools in woodlands around Edmonton. The adults are often very abundant in woods.

- \* punctipennis Say (C.T.) 8. VI-VII.

## ANOPHELINAE Malarial mosquitoes

- \* **Anopheles** maculipennis Meig. (C.T.) 10, 12. IV-VI and X.

In certain years these constitute about 10% of the total mosquito population which enters tents erected near muskeg. There are no authenticated cases of malarial transmission in Alberta.

## CULICINAE

- \* **Aedes** campestris D. & K. (C.T.) 10. VII.
- \* canadensis Theo. (E.H.) 8, 19. VI-VIII.
- \* cataphylla Dyar (E.H.) 10, 19. IV-VIII.
- Usually the earliest mosquito, with the exception of overwintering forms, to appear around Edmonton.
- \* cinereus Meig. (E.H.) 6, 8, 19. V-VIII.
- \* communis deG. (C.T.) 10. V.
- curriei Coq. (? = dorsalis) 19. VI.
- \* dorsalis Meig. (E.H.) 2, 3, 19. VI-VII.

This is a serious pest in irrigated districts.

- \* excrucians Walk. (C.T.) 8, 10, 19. VI-VII.
- \* fitchii F. & Y. (C.T.) 10, 19. IV-VI.
- \* flavescens Müll. (C.T.) 2, 10, 12. IV-VI.
- fletcheri Coq. 5, 6, 8. VII-VIII.
- \* impiger Walk. (E.H.) 8, 10, 19, 20. V-VIII.
- \* intrudens Dyar (C.T.) 10, 19. V-VII.
- \* lazarensis F. & Y. (E.H.) 8, 19. V-VIII.

CULICIDAE—*concluded*.CULICINAE—*concluded*.**Aedes nearcticus** Dyar 19.

- \* **nigromaculis** Lud. (C.T.) 2, 3, 15. VI–VIII.

This is a serious pest in irrigated districts.

**pionips** Dyar 8, 19.

**prodotes** Dyar 19. VII–VIII.

- \* **pullatus** Coq. (E.H.) 19. VII.
- \* **punctor** Kby. (C.T.) 8, 10, 12, 19. VI–VIII.
- \* **spenceri** Theo. (C.T.) 2, 10, 19, 20. VI–VIII.

Larvae of this species are abundant in ditches by roads around Edmonton.

Adults extremely numerous in favorable years. Are vicious biters.

- \* **stimulans** Walk. (C.T.) 10. V.
- \* **trichurus** Dyar (C.T.) 10, 12, 15. V–VI.
- \* **vexans** Meig. (E.H.) 3, 6, 8, 10, 19. VI–VIII.

This is, probably, the most widespread and abundant species in the prairie regions of Alberta.

- \* **Theobaldia alaskaensis** Lud. (E.H.) 6, 10, 19, 20. IV–VII.

Hibernating adults are vicious biters from early in April. Enter houses freely.

**dyari** Coq. 8. VII.

**impatiens** Walk. 8, 12. V–VIII.

- \* **incidens** Thom. (E.H.) 19. VII.
- \* **inornatus** Will. (E.H.) 2, 10, 15, 19. VIII–XI.
- \* **Culex tarsalis** Coq. (C.T.) 3, 19. VII.

## CECIDOMYIDAE. Gall midges

Very little attention has been paid to this Family of minute flies. A small number have been collected in the field, but we have found no specialist who is willing to name them for us. The only named specimens are those which have been bred from readily identified galls.

- \* **Rhabdophaga strobiloides** Walsh. (C.C.) 3, 10. IV.

Cone gall of willows. Very common.

- \* **Diarthronomyia hypogaea** Lw. (E.S.) 10. Bred.

"Chrysanthemum midge". First observed in Alberta in 1929. Now widespread in greenhouses.

**Phytophaga destructor** Say (?) 6.

About 1916, it was recorded at Ottawa, that "flax seeds" had been found in stems of winter wheat from Pincher Creek, Alta. There has been no sign of this insect in subsequent years.

- \* **rigida** O.S. (C.C.) 10, II–III. Bred.

Stem gall of willows.

- \* **Contarinia virginianae** Felt (E.S.) 10, 12. VII.

Forms galls from fruit of choke-cherries.

## SCIARIDAE. Dark-winged fungus gnats

- \* **Eugnoriste occidentalis** Coq. (E.S.) 15. VII.

- \* **Sclara caldaria** Lint. (C.C.) 2. XII.

- \* **coprophila** Lint. (E.S.) 10. IX–III.

A serious mushroom pest around Edmonton.

## MYCETOPHILIDAE. Fungus gnats

The University collection contains long series of flies belonging to this Family. We have attempted to name the more characteristic species ourselves since we have found no specialist who is willing to identify material in this Family.

## BOLITOPHILINAE

- \* **Bolitophila** *disjuncta* Lw. (E.S.) 10. VI.  
*recurva* Garr. 19.

## CEROPLATINAE

- \* **Asindulum** *montanum* Roed. (E.S.) 10. VIII.
- \* **Platyura** *fascipennis sagax* Joh. (E.S.) 12. VI.

## MACROCERINAE

- Macrocera** *variola* Garr. 19.

## SCIOPHILINAE

- Mycomya** *ampla* Garr. 19. VII.
- \* *imitans* Joh. (E.S.) 10. VIII.
- \* *littoralis* Say (E.S.) 10. VII-IX.
- \* *frequens* Joh. (E.S.) 10. VIII.
- \* *obliqua* Say (E.S.) 10. VIII.
- \* *tantilla* Lw. (E.S.) 10. VIII-IX.
- \* **Sciophila** *glabana* Joh. (E.S.) 10. VII-VIII.  
*neohebes* Garr. 19.

## MYCETOPHILINAE

- \* **Leia** *striata* Will. (E.S.) 10, 15. VI-IX.
- \* *winthemii* Leh. (E.S.) 10. VIII.
- \* **Docosia** *nitida* Joh. ? (E.S.) 10, 12. VI.
- \* **Exechia** *obediens* Joh. (C.C.) 10. X.
- \* **Allodia** *bella* Joh. (E.S.) 10. V.
- \* **Dynatosoma** *nigrina* Joh. (C.C.) 10. V.
- \* **Phronia** *venusta* Joh. (C.C.) 10. X.
- Mycetophila** *alberta* Curr. 10. V.
- \* *punctata* Meig. (E.S.) 15.
- \* **Sceptonia** *nigra* Meig. (E.S.) 10. V.

## BIBIONIDAE. March flies

- \* **Hesperinus** *brevifrons* Walk. (D.H.) 8, 10, 15. VI.
- \* **Plecia** *heteroptera* Say (D.H.) 1, 10. V-IX.
- \* **Dilophus** *caurinus* L. (C.C.) 18. VII.
- \* *sectus* McA. (D.H.) 10. VIII.  
*serraticollis* Walk. 19. IX.
- \* *stigmaterus* Say (D.H.) 3, 5, 10. VII-VIII.
- \* **Biblio** *albipennis hirtus* Lw. (C.C.) 2, 3, 10, 18. V-VI.

Larvae sometimes excessively abundant in soil which contains much decaying vegetable matter, such as roots of wild roses in recently broken land. Also in manure. Occasionally damage earthed-up celery, and have been found swarming in undug potatoes. Probably not a primary pest of latter.

- \* *carri* Curr. (C.C.) 2. V-VI.  
*fraternus* Lw. 3. IV.
- \* *fumipennis* Wlk. (D.H.) 8. VII.

**BIBIONIDAE—concluded.**

- \* **Biblio holti** McA. (D.H.) 8, 10, 18. V–VIII.  
obscurus Lw. 19. IX.  
simplicis Curr. 19, 20. VII–VIII.
- \* **slossonae** Ckll. (D.H.) 10. IX and V.  
Adults appear in swarms in woodlands in late autumn.
- \* **tenuipes** Coq. (D.H.) 3. V.
- \* **variabilis** Lw. (D.H.) 8, 15, 18. VI–VIII.
- \* **xanthopus** Wied. (D.H.) 20. VII.

**SCATOPSIDAE. Minute black scavengers**

- \* **Aspistes analis** Kby. (C.C.) 15, 19. VI–VII.
- Scatopse pygmaea** Lw. 3. VII.
- \* **Rhegoclema atrata** Say (E.S.) 10. X.

**STRATIOMYIDAE. Soldier flies****BERIDINAE**

- \* **Beris annulifera** Big. (E.S.) 8, 10. VI–VII.  
canadensis Cress. 18. VII.
- \* **viridis** Say (E.S.) 10. IV–VI.

**CLITELLINAE**

- Euparyphus latilimbatus** Curr. 12, 18. VII.
- \* **limbiventris** Will. (C.C.) 2. VI.
- \* **octomaculatus** Curr. (E.S.) 8. VI.  
quadrimaculatus Cress. 19. V–VI.
- \* **Nemotelus arator** Mel. (E.S.) 10, 19. VII–VIII.
- \* **canadensis** Lw. (C.C.) 3, 9, 19. VI–VII.

**STRATIOMYINAE**

- \* **Stratiomys apicula** Lw. (C.C.) 3, 8, 20. VI.
- \* **badia** Wlk. (C.C.) 10. VI–VIII.
- \* **barbata** Lw. (C.C.) 18, 19, 20. VI–VIII.
- \* **bruneri** Joh. (E.S.) 3, 10. VIII.
- \* **laticeps** Lw. (E.S.) 12, 18, 19. VII.
- \* **lativentris** Lw. (C.C.) 18. VII.
- \* **melanostoma** Lw. (C.C.) 20. VII.
- \* **nevadae** Big. (C.C.) 2, 6. V–VI.
- \* **normula** Lw. (C.C.) 2, 3, 8, 9. VI–VII.
- \* **unilimbata** Lw. (E.S.) 3, 8, 12. VI.
- \* **Odontomyia hoodiana** Big. (C.C.) 10, 19. V–VIII.
- \* **inaequalis** Lw. (E.S.) 3. VIII.
- \* **interrupta** Oliv. (C.C.) 10. V.
- \* **nigrirostris** Lw. 2, 19. VII–VIII.
- \* **pubescens** Day. (E.S.) 3, 15. VI–VII
- similis** Joh. 18.
- varipes** Lw. 12, 19. VI and IX.
- \* **vertebrata** Say (E.S.) 10. VII.
- \* **virgo** Wied. (E.S.) 8, 10. VII.

**SARGINAE**

- Pedicella cuprarius** L. "Alberta".
- \* **decorus** Say (J.J.) 6, 10, 18. VIII.
- \* **viridis** Say (C.C.) 2, 3, 6, 10, 18. V–IX.
- \* **Microchrysa polita** L. (C.C.) 3, 8–10, 12. VI–VII.

## COENOMYIIDAE.

\* *Arthropeas magna* Joh. (C.C.) 6.

Two or three larvae, which key out in Malloch's tables to *Coenomyia ferruginea*, have been taken from the soil in Edmonton. All have refused to feed on Coleopterous, Lepidopterous, and Dipterous larvae, which were offered to them dead and alive, and they died in captivity.

## TABANIDAE. Gad flies, Deer flies, Breeze flies

\* *Chrysops carbonaria* Walk. (A.S.) 10. VI.\* *discalis* Will. (A.S.) 2, 3. VII.

This species, implicated in the transmission of Tularemia, appears to be confined to the driest portion of Alberta. Two or three cases of this disease which have occurred in the northern part of the province do not appear to have been contracted from an insect bite.

\* *excitans* Walk. (A.S.) 12, 14. VI.\* *frigida* O.S. (A.S.) 10. VI-VII.\* *fulvaster* O.S. (C.C.) 2. VII.\* *furcatus* Wlk. (A.S.) 19, 20. VII.\* *mitis* O.S. (A.S.) 1, 3, 8, 10. VI-VII.\* *nigripes* Zett. (A.S.) 10. VII.\* *pertinax* Wlk. (A.S.) 18. VII.\* *proclivis* O.S. (A.S.) 20. VII.\* *Haematopota americana* O.S. (A.S.) 6, 10. VII-VIII.

This gad fly is rather rare in Alberta, though it is apparently abundant elsewhere on the prairie.

\* *Tabanus astutus* O.S. (A.S.) 10, 16, 20. VII-VIII.\* *affinis* Kby. (A.S.) 3, 16. VI-VII.\* *canadensis* Curr. (C.C.) 1. VII.

*centron* Mart. (? = *rhombicus*) 12, 13. V-VI.

*cristatus* Curr. 20. VII.

\* *epistatus* O.S. (A.S.) 10, 12. V-VI.

*fratellus* Will. 19. VIII.

\* *gracilipalpis* Hine (A.S.) 12. VI.\* *hirtulus* Big. (A.S.) 3, 19. VI.\* *illotus* O.S. (A.S.) 8, 10, 15. VI.\* *laniferus* McD. (A.S.) 19, 21. VII-IX.\* *lasiophthalmus* Macq. (A.S.) 12, 20. VI-VII.\* *liorhinus* Phil. (A.S.) 10. VIII.\* *metabolus* McD. (A.S.) 10, 12, 14, 20. V-VI.\* *nivosus* O.S. (A.S.) 10. VI.\* *nudus* McD. (A.S.) 10, 12, 15. VI.

*phaenops* O.S. 19, 20. VII.

\* *reinwardtii* Weid. (A.S.) 3, 6. VII.\* *rhombicus* O.S. (A.S.) 6, 19, 20. VII-VIII.\* *osburni* Hine (C.C.) 18, 19. VII.\* *rupestris* McD. (E.S.) 6, 12. VI.\* *septentrionalis* Lw. (A.S.) 6, 8, 10. VI-VIII.

*sonomensis* O.S. "Alberta".

\* *sexfasciatus* Hine (C.C.) 16. VII.

*trepidus* McD. "Alberta".

\* *typhus* Whit. (A.S.) 12, 16. VI-VII.\* *zonalis* Kby. (A.S.) 16, 19. VII.\* (*Atylotus*) *insuetus* O.S. (A.S.) 6, 20. VII.

## RHAGIONIDAE. Snipe flies

- \* **Symphoromyia atripes** Big. (E.S.) 18, 19. VII.

This species, with *S. hirta*, is frequently a serious nuisance in the National Parks of the Rocky Mountains. The bite is not painful but the flies are most persistent in attacking, both in the shade and in the open.

- \* *cinerea* Joh. (E.S.) 18. VII.
- \* *hirta* Joh. (C.C.) 18, 19. VII.
- \* *johnstoni* Coq. (C.C.) 18. VII.
- \* *montana* Ald. (C.C.) 20. VII.
- \* *pachyceras* Will. 19. VII.
- \* **Chrysopilus quadratus** Say (C.C.) 9, 10, 16. VI-VII.
- \* **Ptiolina alberta** Leon. 19. VII.

## ASILIDAE. Assassin flies

## DASYPOGONINAE

- \* **Comantella fallai** Back. (C.C.) 2. II-III.
- \* *rotgeri* James. 2. III-V and X.
- \* **Nicocles punctipennis** Mel. 2. IV.
- \* **Stenopogon gratus** Lw. (F.C.) 3. VIII.
- \* *inquinatus* Lw. (C.C.) 2, 6. VII-IX.
- \* *obscuriventris* Lw. (E.S.) 7. VI.
- \* *pumilis* Coq. (C.C.) 2. VII.
- \* **Neopogon (= Stichopogon) argenteus** Say. 2. IX.
- \* **Lasiopogon aldrichi** Mel. 19. VII.
- \* *cinereus* Cole (C.C.) 18, 19. VII-VIII.
- \* *quadrivittatus* Jones 2. V.
- \* **Eucyrtopogon albibarbis** Curr. (F.C.) 2, 6, 10. IV-VI.
- \* *calcarata* Curr. (C.C.) 6, 19. V and X.
- \* *comantis* Curr. (C.C.) 2. IV.
- \* *spinigera* Curr. (F.C.) 6, 12. V-VI.
- \* **Cyrtopogon albitarsis** Curr. 18, 19. VII.
- \* *albovarians* Curr. 19. VII.
- \* *bimacula* Wlk. (F.C.) 6, 10, 15, 20. V-IX.
- \* *dasyllis* Will. 19.
- \* *linneotarsus* Curr. 19. VIII.
- \* *montanus* Lw. 19.
- \* *nebulo* O.S. 19. III.
- \* *nugator* O.S. 19. VI-VII.
- \* *praepes* Will. 6, 19.
- \* *sansoni* Curr. 19. VII.
- \* *vulneratus* Mel. (F.C.) 10. VII.
- \* *willistoni* Curr. (C.C.) 6, 18, 19. VI-VII.
- \* **Holopogon albopilosa** Curr. (C.C.) 3. VII.

## LAPHRINAE

- \* **Pogonosoma ridingsi** Cress. 18. VII.
- \* **Bombomima colombia** Walk. (F.C.) 19. VI.
- \* *insignis* Bnks. (C.C.) 10. VII.
- \* *partitor* Bnks. 19. VII.
- \* *posticata* Say (C.C.) 12. VIII.
- \* **Laphria aeatus** Wlk. 12. VI-VII.
- \* *aimites* Mtlf. 10. IX.
- \* *ferox* Will. 19. X.
- \* *gilva* L. (C.C.) 9, 19. VII.

**ASILIDAE—concluded.****LAPHRINAE—concluded.**

- \* **Laphria** janus McA. (C.C.) 10, 19. V–VIII.  
pubescens Will. 19. VIII.
- \* **sadales** Wlk. (F.C.) 20. VII.  
xanthippe Will. 19. VI.

**ASILINAE**

- \* **Erax** bicaudatus Hine (C.C.) 2, 3. VII–VIII.
- \* **subcupreus** Sch. (C.C.) 2. VI.
- Rhadiurgus** cacopillogus Hine 2. IX.
- \* **Asilus** auriannulatus Hine (C.C.) 18. VII.  
nitidifacies Hine 19. VII.
- Machimus** antimachus Walk. 3. VII.
- \* **callidus** Will. (C.C.) 3, 10, 18, 19. VII.
- \* **erythrocnemis** Hine (C.C.) 1–5. VII–VIII.
- \* **occidentalis** Hine (C.C.) 19. VIII.
- \* **paropus** Walk. (C.C.) 10. VII.
- \* **snowi** Hine (C.C.) 3. VIII.

**THEREVIDAE. Stiletto flies**

- \* **Thereva** cingulata Krob. (F.C.) 18. VII.
- \* **cockerelli** Cole (F.C.) 8, 10. VI–VIII.
- \* **duplicis** Coq. (F.C.) 1, 6, 8, 10, 15. IV–VIII.  
Larvae are very abundant in cultivated fields. It is stated that they feed on wireworms and small cutworms, but we have failed to induce them to attack either.  
mcdunnoughi Cole 20. VII.  
strigipes Lw. 19.
- \* **Psilocephala** albertensis Cole (C.C.) 2, 3. IV–VI.
- \* **aldrichi** Coq. (F.C.) 1–3, 10, 18. VI–VIII.  
baccata Coq. 2. V.  
coquilleta Ald. 3. VII.  
limata Coq. 18. VII.
- \* **munda** Lw. (C.C.) 1, 6, 9, 10, 15, 19. V–VII.

**BOMBYLIIDAE. Bee flies**

- \* **Anthrax** analis Say (C.C.) 10. VIII.  
Reported to be a parasite of tiger beetles.
- \* **oedipus** Fall. (= *irrorata*) (F.C.) 8, 10, 18. VI–VII.
- \* **plesia** Curr. (F.C.) 18. VII.
- \* **varia** Fall. (C.C.) 7, 18. VI–VII.
- \* **Exoprosopa** caliptera Say (F.C.) 3, 10. VI–VII.
- \* **decora** Lw. (C.C.) 1–3. VI–VIII.  
dorcadion O.S. 18. VII.
- \* **Thyridanthrax** alpha O.S. (C.C.) 2. IX.
- \* **bigradata** Lw. (C.C.) 2. V.
- \* **halcyon** Say (C.C.) 2, 10. VII–VIII.
- \* **lateralis** Say (C.C.) 1, 3, 6, 10. VI–VIII.
- \* **muscaria** Coq. (C.C.) 2. VIII.
- \* **sackenii** Coq. (C.C.) 3, 6. VIII–IX.
- \* **salebrosus** Paint. (C.C.) 2. VI.
- \* **tegminipennis** Say (F.C.) 2, 3. VIII–IX.

**BOMBYLIIDAE—concluded.**

- \* **Thyridanthrax** willistoni Coq. (C.C.) 2, 3, 6. VII–IX.

The genus *Thyridanthrax* has recently been derived from *Villa* as a valid genus. All the species in our collection were determined as *Villa*. We have placed in the former genus all species represented in the collection which appear to belong there, and have left all doubtful or unseen species in *Villa*.

- \* **Villa** alternata Say (C.C.) 1, 6, 10. VII–X.
- \* eumenes O.S. (C.C.) 3. VII.
- \* fulviana Say (C.C.) 3, 6, 10, 15, 18–20. VII–VIII.  
nigricauda Lw. 19. VII.
- \* hypomelas Mg. (F.C.) 1. VII.
- \* lepidota O.S. (C.C.) 3. V.
- \* molitor Coq. (F.C.) 4. VII.
- \* morio L. (= morioides) (C.C.) 6–10, 18, 20. V–VIII.
- \* mucorea Lw. 3. VII.  
pretiosa Coq. 18. VII.
- \* sinuosa Wd. (F.C.) 3, 6, 8, 10, 18. VI–IX.
- \* syrtis Coq. (C.C.) 2. VII.
- \* **Toxophora** pellucida Coq. (F.C.) 2. VIII.
- \* **Bombylius** albocapillus Lw. (C.C.) 2, 6, 19. IV–V.
- \* aurifer O.S. (C.C.) 18. VII.
- \* lancifer O.S. 18. VII.
- \* major L. (C.C.) 2, 10, 14. IV–V.

This very common species is recorded as a parasite of solitary bees.

- pulchellus Lw. 10. V.
- \* pygmaeus Fabr. (F.C.) 8, 10. V–VI.
- \* **Anastoechus** nitidulus F. (C.C.) 2, 6, 10. VIII.
- Systoechus** solitus Walk. 3. VII.
- \* oreas O.S. (F.C.) 2, 3, 7. VI–VIII.
- \* vulgaris Lw. (C.C.) 1–5. VII.

The most important predator on the eggs of grasshoppers.

- \* **Conophorus** nr. nigripennis Lw. (F.C.) 18. VI.
- \* **Lordotus** gibbus Lw. (C.C.) 2. VIII.

**CYRTIDAE. Spider parasites**

Dr. F. C. Cole, who has the material from the University collection for identification, states that there are additional species represented.

- \* **Ogcodes** incultus Lw. (F.C.) 18. VII.
- \* marginatus Cole (C.C.) 1. VII.
- Acrocera** bulla West. 2. X.
- \* convexus Cole (F.C.) 10. VII.
- \* fasciata Wied. (F.C.) 2, 10. VII.

**EMPIDAE. Dance flies, or Balloon flies**

A large number of species belonging to this Family are represented in the University collection. During recent years we have failed to find any specialist who is willing to name these for us. We have attempted to name a few which appear to be readily recognized from published descriptions, but the bulk of the collection is unnamed.

**EMPIDAE—concluded.****HYBOTINAE**

- \* **Hybos** triplex Walk. (E.S.) 10. VI.
- \* **Ocydromia** glabricula Fall. (E.S.) 10, 15. VI–VIII.
- \* **Oedalla** ohioensis Mel. (E.S.) 10. VIII.

**EMPINAE**

- \* **Hormopeza** bullata Mel. (E.S.) 10. VIII.  
nigricans Lw. 19. VII.
- Hilaria** auripes Curr. 19. VII.  
garretti Curr. 19. VI–VII.  
granditarsis Curr. 19. VII.  
rufopuncta Curr. 19. VII.
- \* **Hesperempis** mabelae Mel. (E.S.) 10. VI.
- \* **Empis** brachysoma Coq. (C.C.) 18. VII.
- \* **Rhamphomyia** flexuosa Coq. (C.C.) 5, 10, 18, 20. V–VII.  
This appears to be the most abundant and widespread species in Alberta.  
The Genus is well represented by other species, however.

**HEMERODROMINAE**

- Clinocera** simplex Lw. 19. VIII.

**TACHYDROMINAE**

- \* **Tachypeza** clavipes Lw. (E.S.) 15. VI.
- \* winthemi Zett. (E.S.) 15, 20. VI–VII.
- Phoneustica** maculipennis Walk. "Alberta" VI–VIII.
- \* **Platypalpus** aequalis Lw. (C.C.) 3, VI–VII.  
pectinator Mel. 19.  
perimerus Mall. 3. VI.
- \* **Drapetis** scissa (E.S.) 10. VI.  
(**Eudrapetis**) facialis Mel. 2.

**DOLICHOPODIDAE. Long-headed flies**

- Dolichopus** adequatus V.D. 19. VI.
- \* aeratus V.D. (E.S.) 3, 19. VII.
- \* affluens V.D. (E.S.) 10. VII.  
albertensis Curr. 19. VI–VII.
- \* albiciliatus Lw.? (C.C.) 3. VII.
- \* albicoxa Ald. (E.S.) 19, 20. VII–VIII.
- \* amphericus M. & B. (C.C.) 8, 10, 18. VI–VII.
- \* barbicauda V.D. (E.S.) 10. V.
- \* bakeri Cole (E.S.) 8, 19. VI–VII.
- \* bifractus Lw. (C.C.) 1–3, 8, 20. VI–VIII.  
breviciliatus V.D. 19. VIII.
- \* brevipennis Meig. (C.C.) 3, 8, 15, 19, 20. VI–VII.
- \* canadensis V.D. (C.C.) 2, 8, 10, 15. VI.
- \* coloradensis Ald. (E.S.) 10, 19. VI–VII.  
conspetus V.D. 3. V.
- \* discifer Stan. (E.S.) 10, 12, 20. VI–VII.  
diversipennis Curr. 20. VII.
- \* flaviciliatus V.D. (E.S.) 7. VII.  
flagellitenens Wheel. 6. VIII.
- \* frontalis V.D. (E.S.) 10, 20. VII.
- \* fulvipes Lw. (E.S.) 3, 8, 19. VII.  
gratus Lw. 19. V–VI.

**DOLICHOPODIDAE—continued.**

- \* **Dolichopus** lobatus Lw. (C.C.) 8, 10, 12. VI–VII.
- \* longicornis Stan. (E.S.) 12. VI.
- \* longimanus Lw. (E.S.) 8, 10, 12, 15. VI–VII.
- maculitarsis V.D. 3. V.
- manicula V.D. 19. VII.
- \* nigricornis Mg. (C.C.) 10. VII.
- nigrilineatus V.D. 10, 20. VII.
- nigrimanus V.D. 19. VII.
- \* nudus Lw. (E.S.) 19, 20. VII–VIII.
- \* obcordatus Ald. (C.C.) 3, 8, 10, 19, 20. V–VII.
- \* omnivagus V.D. (E.S.) 10, 19. VII–VIII.
- paluster M. & B. 19. V.
- pernix M. & B. 3, 19. VI–VII.
- pilatus V.D. 19. VIII.
- \* plumipes Scop. (E.S.) 3, 8, 18. VI–VII.
- plumosus Ald. 18. VII.
- pollex O.S. 19. VII.
- \* procerus V.D. (E.S.) 3, 10, 19. VI–VIII.
- \* ramifer Lw. (C.C.) 2, 3. IV–VI.
- \* remipes Wahl. (E.S.) 10. VII.
- \* renidescens M. & B. (C.C.) 3, 8, 10, 15, 19. V–VIII.
- \* robertsoni Curr. (C.C.) 3. VIII.
- \* sincerus Mel. (E.S.) 20. VII.
- \* socius gladius V.D. (E.S.) 8. VII.
- \* speciosus V.D. (C.C.) 18, 19. VII–VIII.
- \* splendidulus Lw. (C.C.) 8, 10, 15. VI–VII.
- \* splendidus Lw. (C.C.) 2, 10, 15. V–VII.
- sufflavus V.D. 19.
- \* stenhammari Zett. (E.S.) 15. VI.
- uxorcula V.D. 19. VII.
- vanduzeei Curr. 19.
- \* variabilis gracilis Ald. (F.M.) 10, 19. VII.
- Liancalus** hydrophilus Ald. 19. IX–X.
- \* **Medeterus** trisetosus V.D. (E.S.) 18, 19. VI.
- Tachytrechus** sanus O.S. 18. VI–VII.
- Hercostomus** unicolor Lw. 18. VII.
- \* **Neurilgona** ciliata V.D. (C.C.) 8, 10. VII.
- nigrimanus V.D. 19. VII.
- ornatus V.D. 10. VI.
- planipes V.D. 18. VII.
- \* **Scellus** amplus Curr. (C.C.) 3, 18. VII.
- avidus Lw. 6.
- \* filiferus Lw. (C.C.) 1, 6, 18. VI–VII.
- \* monstrosus O.S. (E.S.) 3. VII.
- vigil O.S. 3, 19.
- Hydrophorus** aestuum Lw. 3. VI.
- algens Wheel. 19. VII–IX.
- altivagus Ald. 3. VII.
- ampullaceus V.D. 19. VIII.
- cerutias Lw. 4. VIII.
- fulvidorsum V.D. 3.
- gratiosus Ald. 3. V.
- \* purus Curr. (E.S.) 3, 4. VIII.

**DOLICHOPODIDAE—concluded.**

- Rhaphium** campestris Curr. 19. VII.  
 crassipes Mg. 19. VIII.  
 effilatum Wheel. "Alberta".  
 femoratum V.D. 18, 19. V-VIII  
 latifacies V.D. 19. VII.  
 longibara V.D. 19. VI-VII.  
 spinitarsis Curr. "Alberta".  
**Diaphorus** snowii V.D. 19. VIII.  
**Parasyntormon** emarginicornis Curr. 19. VII.  
**Chrysotus** hirtipes V.D. "Alberta" VI-VII.  
**Sympycnus** cuprinus Wheel. 19. VIII.  
 marcidas Wheel. 19. VII-VIII.  
**Pelastoneurus** vagans O.S. 3. VI.  
 \* **Argyra** bimaculata V.D. (E.S.) 12. VI.  
 \* robusta Joh. (E.S.) 10. VI.

**LONCHOPTERIDAE.** Pointed-wing flies

- \* **Lonchoptera** borealis Curr. (E.S.) 10. VI.  
 \* occidentalis Curr. (E.S.) 10. VI.  
 \* uniseta Curr. (E.S.) 10. IV.  
 These flies have been swept from rushes near lakes.

**PHORIDAE.** Hump-back flies

We have about a dozen species in the collection, among which we have identified with a fair degree of certainty, the following:

- \* **Hypocera** clavata Lw. (E.S.) 10. VI.  
 \* femorata Mg. (E.S.) 8, 10. VI.  
 \* **Gymnophora** arcuata Mg. (E.S.) 10. VII.  
 \* **Megaselia** rufipes Mg. (E.S.) 10, 19. V-VI.  
 \* **Trineura** velutina Mg. (E.S.) 10. V.

**PLATYFEZIDAE.** Flat-footed flies

Several species in this Family have been taken walking on foliage of bushes near water. We have been unable to find a specialist to name them for us, but two key out rather readily to the following species.

- \* **Agathomyia** vanduzeei Joh. (E.S.) 10. IX.  
 \* **Callimya** venusta Snow (E.S.) 12. VI.

**PIPUNCULIDAE.** Big-headed flies

- \* **Pipunculus** albofasciatus Hough (E.S.) 15. VI.  
 exilis Mall. 2.  
 \* houghi Kert. ? (E.S.) 10. VI.  
 inconspicuus Mall. 2. X.  
 occidentalis Mall. 2. X.  
 \* stricklandi Curr. (E.S.) 10, 18. VII.  
**Nephrocerus** daeckeri Joh. 10. VI.

**SYRPHIDAE.** Flower or Hover flies**CERIOIDINAE**

- \* **Ceriodes** abbreviata Lw. (C.F.) 10. VII-VIII.

SYRPHIDAE—*continued*.

## MICRODONTINAE

**Microdon** albipilis Curr. 3. VIII.

\* cothurnatus Big. (C.F.) 10, 12. V-VI.

\* piperi Knab (C.C.) 18. VII.

## VOLUCELLINAE

\* **Copestylum** caudatum Curr. (C.C.) 2, 3. V-VII.

\* **Volucella** bombylans facialis Will. (E.S.) 8, 10, 20. VII-VIII.

\* lateralis Joh. (E.S.) 10. VII.

\* rufomaculata Jones (C.C.) 6. V.

\* satur O.S. (C.C.) 2. VIII.

## SYRPHINAE

**Chrysotoxum** coloradense Green "Alberta".

\* derivatum Wlk. (C.C.) (? = ventricosum) 10, 16, 18, 19. VII-VIII.

pubescens Lw. 10. VII.

ypsilon Will. (? = occidentale) 19.

\* **Paragus** bicolor Fab. (C.C.) 2, 18. VI-VII.

\* tibialis Fall. (C.C.) 2, 3, 6, 20. V.

\* **Leucozona** leucorum L. (C.F.) 10. VII.

\* americana Curr. (C.C.) 10. VII.

\* **Eupeodes** volucris O.S. (C.C.) 1-3, 6. V-VII.

\* **Scaeva** pyrastris L. (C.C.) 2, 3, 6, 10, 18, 19. VI-X.

\* **Syrphus** amalopus O.S. (C.C.) 18, 19. VI-VII.

\* arcuatus Fall. (J.A.) (? = perplexus or lapponicus) 19. VII.

attenuatus Hine 19.

\* bigelowi Curr. (C.C.) 10, 18, 20. VII-VIII.

insolitus Osb. 18. VI.

\* lapponicus Zett. (C.C.) 18. VII-VIII.

\* laxa O.S. (C.C.) 18. VII.

\* opinator O.S. (C.C.) 3, 6, 18, 19. V-IX.

\* osburni Curr. (J.A.) (? = amalopus) 10, 18. V.

pacifica Lw. "Alberta".

palliventris Curr. 20. VI.

\* perplexus Osb. (J.A.) 10, 18. V-VIII.

\* rectus O.S. (C.C.) 3, 10, 20. IV and VII.

\* ribesii L. (J.A.) 10, 12. V-VIII.

\* vittafrons Shn. (C.C.) 10. VIII.

\* torvus O.S. (C.C.) 3. V.

\* umbellatum O.S. (J.A.) 10. VI.

\* venabesi Curr. (= nitens) (C.C.) 6, 10. V and VIII.

\* vitripennis Mg. (C.C.) 3, 10. VII-VIII.

\* wiedemanni Joh. (C.C.) 3. VII.

\* **Epistrophe** albipunctatus Curr. (C.C.) 10. VIII.

contumax O.S. 19. VI-VII.

fisheri Walt. (C.C.) 10. VIII.

\* garretti Curr. (C.F.) 10, 19. VI.

\* grossulariae Mg. (C.C.) 18. VII.

\* melanis Curr. (C.C.) 18. VII.

nigrifascies Curr. 19. VII.

rectoides Curr. (C.C.) 18. VII.

\* sexpunctatus Walk. ? (C.C.) 10. VII.

\* sodalis Will. (C.C.) 18, 19. V-VII.

subfasciatus Curr. 19.

SYRPHIDAE—*continued*.

- \* **Epistrophe** *tenuis* Osb. (C.C.) 10. VI.
- \* **Mesogramma** *marginata* Say (C.F.) 3, 10, 15. VII.
- \* **Sphaerophoria** *cylindrica* Say (C.F.) 3, 8, 10, 12, 15. V-VIII.
- \* *robusta* Met. (C.C.) 2, 3, 15, 18. V-VIII.
- \* **Pyrophaena** *granditarsis* Forst. (C.C.) 10. VIII.
- \* **Platycheirus** *angustatus* Zett. (C.F.) 8, 10. VII.
- \* *erraticus* Curr. (C.F.) 10. V.
- \* *hyperboreus* Staeg. (C.C.) 12. VIII.
- \* *immarginatus* Zett. (C.F.) 10. VII-VIII.
- \* *modestus* Ide (C.F.) 10. VI.
- \* *nodosus* Curr. 19. VI.
- \* *peltatoides* Curr. ? (C.F.) 8. VI.
- \* *peltatus* Mg. (C.C.) 10, 18. VII-VIII.
- \* *perpallidus* Verr. (C.F.) 3, 8, 10. V-VII.
- \* *podagratus* Fab. 19. V-VII.
- \* *quadratus* Say (E.S.) 3. VI.
- \* *scutatus* Mg. (C.F.) 10, 19. IV-VI.
- \* **Melanostoma** *angustatum* Will. (C.C.) 18. VII.
- \* *caerulescens* Will. (C.C.) 1, 3, 6. V-VI.
- \* *nr. chaetopoda* Dav. (C.F.) 12. VI.
- \* *chilosia* Curr. 19.
- \* *fallax* Curr. 19. V.
- \* *monticola* Jones (C.F.) 10. V.
- \* *nr. obscurum* Say (C.F.) 12. VI.
- \* *pallitarsis* Curr. (C.F.) 10, 12. VI-VII.
- \* *pictipes* Big. (C.F.) 8, 10. V-VII.
- \* *rufipes* Will. (C.C.) 18. VII.

## CHEILOSINAE

- \* **Cartosyrphus** *canadensis* Shn. (C.C.) 18. VI-VII.
- \* *kincaidi* Shn. (C.F.) 10. V-VI.
- \* *laevis* Big. 19. VII.
- \* *luctus* Snow 19. VI.
- \* *plutonia* Hunt. (C.C.) 10, 18. VII.
- \* *sialia* Shn. (C.F.) 10, 12. V-VI.
- \* *tristis* Lw. (C.C.) 10, 18, 20. V-VII.
- \* **Cheliosia** *bicolorata* Shn. (C.F.) 10. V.
- \* *ferruginea* Lov. 1. V.
- \* *florella* Shn. (C.F.) 8, 10. V-VI.
- \* *hesperia* Shn. 10. VI.
- \* *lasiophthalma* Will. (C.C.) 1, 10. V.
- \* *nigroapicata* Curr. (C.C.) 1, 10. V-VI.
- \* *nigrovittata* Lov. 19. V-VI.
- \* *orilliaensis* Curr. (C.F.) 10. VII.
- \* *robusta* Hine (C.C.) 6, 18. VI-VII.
- \* *variabilis* Panz. (C.C.) 18. VII.
- \* **Pipizia** *femoralis* Lw. (C.F.) 10, 12, 20. V-VII.
- \* *macrofemoralis* Curr. (C.C.) 18. VII.
- \* *oregona* Lov. (C.C.) 10, 18. VI.
- \* *quadrifaculata* Panz. (C.C.) 18, 19. VI-VII.
- \* *vanduzeei* Curr. ? (C.F.) 8, 10. VI.
- \* **Cnemon** *auripleura* Curr. (C.C.) 3, 6. VI-VII.
- \* *calcarata* Lw. ? (C.F.) 10. V.

SYRPHIDAE—*continued*.

- Cnemodon nigricornis** Curr. 19. VI.  
     rita Curr. 18. VII.
- \* **Pipizella apisaon** Wk. (C.F.) 8, 10. VI.
  - \* **recedens** Wk. (C.F.) 8, 10. VII.
  - \* **Chrysogaster parva** Sch. (C.C.) 3, 8. V-VI.
  - \* **pictipennis** Lw. (C.F.) 8, 10. V-VI.
  - \* **pulchella** Will. (C.F.) 8, 10, 20. V-VIII.
  - \* **Neoscia conica** Curr. ? (C.F.) 10, 19. V-VII.  
     metallica Will. 19. V-VI.  
     sphaerophoria Curr. 19. V-VI.
  - \* **unifasciata** Curr. ? (C.F.) 10, 12. VI-VIII.
  - \* **Hammerschmidtia ferruginea** Fall. (C.C.) 18. VII.
  - \* **Rhingia nasica** Say (C.C.) 10, 15, 16, 18. VI-VII.
  - \* **Brachyopa notata** O.S. (C.F.) 10. V.
  - \* **vacua** O.S. (C.F.) 12. V.

## XYLOTINAE

There appears to be some difference of opinion among authorities regarding the generic designation of several species included in this sub-family. We have attempted to follow Curran, but we fail to see how *Eumerus strigatus* can be included in the genus *Heliophilus* where we have placed it, on his authority.

- \* **Cynorhina armillata** O.S. (C.C.) 18. VII.
  - \* **garretti** Curr. (C.F.) 8, 19. VIII.
  - \* **nigripes** Curr. (C.F.) 10. VI.
  - \* **Brachypalpus (Xylotodes) parvus** Will. (C.C.) 6, 10. V.
  - \* **rileyi** Will. (C.C.) 10. IV-V.
  - \* **inarmatus** Hunt. (C.F.) 10. VI.
  - \* **pigra** Lov. (C.F.) 12. V.
  - \* **Heliophilus (Xylotomima) curvipes satanica** Big. (C.F.) 8, 10. VI-VII.
  - \* **nemorum americanum** Shn. (C.F.) 10. V.
  - \* **vecors** O.S. (C.F.) 10. V.
  - \* **(Xylota) ejuncida** Say (C.F.) 12. VII.
  - \*      **elongata** Will. (C.F.) 8. VI.
  - \* **flavitibia** Big. (C.F.) 8, 19. VI-VIII.
  - \* **naknek** Hine (C.F.) 8, 10, 16. VI-VIII.
  - \* **subfasciatus** Lw. (C.C.) 8, 10, 18. VI-VII.
  - \* **(Eumerus) strigatus** Fall. (C.C.) 3, 10. V-VII.
- Rather injurious to onions in drier part of the province. Rare around Edmonton.
- \* **Spilomyia quadrifasciata** Say. (C.C.) 2, 15. VII-VIII.
  - \* **Temnostoma apiforme** Fab. (E.S.) 10, 15. VI.
  - \* **bombylans** F. ? (C.F.) 10. V.
  - \* **Tropidia quadrata** Say. (C.F.) 10, 20. VI-VIII.
  - \* **Syritta pipiens** L. (C.C.) 2, 3, 5, 8, 10, 12, 15. V-VIII.

## SERICOMYINAE

- \* **Sericomyia militaris** Wlk. (C.C.) 1, 10, 20. VII-VIII.
- Pyrilis montigena** Hunt 8. IV.
- \* **Arctophila flagrans** O.S. (C.C.) 6, 18, 19. VII-VIII.

## SYRPHIDAE—concluded.

## ERISTALINAE

- \* *Eristalis anthophorinus* Fall (= *montanus*) 1-3, 10, 12. V-VIII.
- \* *bastardi* Mg. (J.A.) (? = *anthophorinus*) 10, 12. VII-VIII.
- \* *brousii* Will. (C.F.) 2, 3, 6, 10. V-IX.
- \* *compactus* Walk. (C.F.) 19, 20. VII.
- \* *dimidiatus* Wd. (C.C.) 10. VIII.
- \* *flavipes* Walk. (C.C.) 3, 5. V and IX.
- \* *hirtus* Lw. (J.A.) (? = *latifrons*) 19. VII-VIII.
- \* *latifrons* Lw. (C.C.) 3, 4, 6, 10, 19. VIII.
- \* *nemorum* L. (C.F.) 15, 19. VI.
- occidentalis* Will. 19.
- \* *rupium* Fab. (C.F.) 10, 12, 20. VI-VII.
- \* *temporalis* Thom. (C.C.) 3, 18. VII-VIII.
- \* *tenax* L. (C.C.) 1-3, 6, 8, 10, 15, 18. VIII-X.
- \* *campestris* Mg. (E.S.) 3. VIII.

*Merodon equestris* Fab. 10. IV.

Determined from Edmonton material by Curran. We have not seen a specimen and have no records of injury.

- \* *Polydontomyia curvipes* Wd. (C.C.) 2, 10. V-VIII.
- \* *Lejops bilinearis* Will. (C.F.) 10. VI.
- \* *perfidiosus* Hunt. (C.F.) 10, 19. VI-VII and X.
- \* *stipatus* Walk. (E.S.) 2, 10. VI-VII.
- \* *Parhelophilus obsoletus* Lw. (C.F.) 10. VI.
- porcus* Walk. 19. VII.

*Elophilus borealis* Staeg. "Alberta".

- \* *hybridus* Lw. (C.C.) 4, 10. V-VIII.
- Several adults were taken from under stones around a grain field in May.
- \* *latifrons* Lw. (C.C.) 2, 3, 6, 10. V-VIII.
- \* *obscurus* Lw. (C.C.) 3, 10, 20. VI-VII.
- stricklandi* Curr. 10. V.
- \* *Asemosyrphus willingi* Sm. (C.C.) 3, 10. V-VIII.

## CONOPIDAE. Thick-headed flies, bee and wasp parasites

- \* *Physocephala affinis* Will. (C.C.) 3. VIII.
- \* *buccalis* V.D. (F.C.) 3. VIII.
- \* *furcillata* Will. (C.C.) 9, 10, 12, 15. VII-VIII.
- \* *marginata* Say. (C.C.) 3. VI.
- \* *Zodion fulvifrons* Say (F.C.) 3, 8. VI-VIII.
- obliquefasciata* Macq. 2, 3, 10. VI-VIII.
- \* *occidentale* Bnks. (C.C.) 3, 18. VI-VIII.
- \* *Myopa longipilis* Bnks. (C.C.) 6. V.
- \* *seminuda* Bnks. (? = *rubida*) (F.C.) 10. V.
- \* *vesiculosa varians* Bnks. (C.C.) 2, 6. V.
- \* *vicaria* Wlk. (C.C.) 9, 10. IV-V and VIII.
- \* *Occemya baroni* Will. (F.C.) 3, 10. V-VII.
- \* *terminalis* V.D. (F.C.) 10.

## OTITIDAE (ORTALIDAE). Pictured-wing flies

- \* *Oedopa capito* Lw. (C.C.) 3. VI.
- \* *Seloptera colon* Lw. (C.C.) 3. VI-VII.
- \* *vibrans* L. (C.C.) 3, 8, 10. VI-VIII.
- \* *Chrysomya demandata* Fall. (C.C.) 3. III and VII.

## OTITIDAE—concluded.

- Pseudotephritis** corticalis Lw. 10. V.
- \* **cribellum** Lw. (E.S.) 3. VII.  
metzi Joh. 10.
- \* **Tritoxa** cuneata Lw. (C.C.) 3, 15, 18. VI-VII.
- \* **Rivellia** flavimana Lw. (C.C.) 2. VII.
- \* **Melieria** obscuricornis Lw. (C.C.) 2, 3. V-VI.
- \* **ochreicornis** Lw. (C.C.) 3, 6, 10. VI-VIII.
- \* **Tetanops** aldrichi Hend. (C.C.) 2, 3, 5-7, 9, 18. V-VIII.  
The sugar-beet root-maggot has become a serious pest in southern Alberta. Damage was first observed in about 1930. Adults have been taken in numbers, since 1916.
- \* **Ceratoxys** (Anacampta) pyrocephala Lw. (C.C.) 2. VII.

## TRUPANEIDAE (TRYPETIDAE). Fruit flies

- \* **Epochra** canadensis Lw. (E.S.) 2, 3, 6, 10, 15. V-VII.  
The currant fruit fly is found wherever currants and gooseberries grow, wild or cultivated. It is an increasingly serious pest of cultivated varieties.
- \* **Zonosema** flavinotata Macq. (F.M.) 10. VI and VIII.
- \* **setulosa** Doane (E.S.) 10, 18. VII.  
**Rhagoletis** pomonella Walsh (? = symphoricarpi Curr.) 3. VII.  
This breeds in *Symphoricarpos* berries in Alberta. As such, it was described as a new species by Curran. Cresson states that it is *pomonella*. We have no records of it attacking apples in Alberta.
- \* **tabellaria** Fit. (E.S.) 10, 15. VI-VII.
- \* **Trypeta** occidentalis Snow (C.C.) 2, 3, 6, 18. VI-VIII.
- \* **Terellia** palposa Lw. (F.M.) 15. VIII.  
Adults taken exclusively from blossoms of "bull-thistle".
- \* **Straussia** longipennis Wd. (C.C.) 3. VI-VII.  
The sunflower maggot is an appreciable pest of sunflowers, grown for silage, in southern Alberta.
- \* **longitudinalis** Lw. 3. (E.S.) VII.
- \* **vittigera** Lw. (E.S.) 8. VI.
- \* **Tephritis** aequalis Lw. (C.C.) 3. VIII.
- \* **albiceps** Lw. (C.C.) 3, 10. VI.
- \* **angustipennis** Lw. (F.M.) 10. VII.  
aldrichi Hine 18. VI.
- \* **murina** Doane (C.C.) 3. VIII.
- \* **pura** Lw. (F.M.) 8, 10, 15. V-VII.
- \* (? = **Euribia**) **clathrata** Lw. (E.S.) 15.
- Stenopa** vulnerata Lw. 19.
- \* **Paracantha** culta Lw. (C.C.) 1, 6. V-VII.
- Eutreta** diana O.S. 2. VIII.
- \* **longicornis** Snow (C.C.) 2. VI-VIII.
- \* **Eurosta** comma Wd. (C.C.) 2. VIII.
- \* **[solidaginis fascipennis** Curr. (C.C.) 10. IV.
- \* **subfascipennis** Curr. (C.C.) 2. V.  
This stem-gall fly of goldenrod is annually very abundant at Edmonton.

## PALLOPTERIDAE.

- \* **Palloptera** albertensis Joh. (C.J.) 6. VII.

## LONCHAEIDAE.

- \* *Lonchaea* laticornis Mg. (E.S.) 18, 19. VII–VIII.
- \*     *polita* Say. (E.S.) 15. VI.
- \*     *ursina* Mall. (E.S.) 10. VII.

## TANYPEZIDAE.

- \* *Tanypeza* picticornis C. & S. ? (G.S.) 10. VI–VII.

## CALOBATIDAE. Stilt-legged flies

- \* *Calobata* mimia Cress. (C.C.) 7, 10. VI.
- \*     *univittata* Walk. (E.S.) 10. VII.

## MICROPEZIDAE.

- \* *Micropeza* lineata V.D. 2.
- \*     *turcata* Twms. 2, 3. (C.C.) VI.

## PIOPHILIDAE. Cheese skippers

- \* *Piophilha* affinis Mg. (C.C.) 18. VI.
- casei* L. (?).

A sample of oats, submitted for a germination test, was heavily infested with "cheese skippers". Correspondence elicited the fact that these oats had been kept in a room where cheese was being made.

## SEPSIDAE. Small scavenger flies

- \* *Nemopoda* cylindrica Fab. (E.S.) 10. VII–VIII.
- \* *Sepsis* pectoralis Macq. (C.C.) 3, 9, 10. VII.
- \*     *signifera* Mel. (C.C.) 2, 15. VI.
- \*     *vicaria* Walk. (E.S.) 10, 15. V–VI.
- \*     *violacea* Mg. (C.C.) 3, 9, 19. VI.
- \*         *similis* Macq. (C.C.) 19. IV–VI.
- \* *Themira* putris L. (E.S.) 2, 10. VII–VIII.

## LAUXANIIDAE, (SAPROMYZIDAE).

- \* *Lauxania* cylindricornis Fall. (C.C.) 2, 3, 10. V–VII.
- \* *Camptoprosopella* vulgaris Fitch (G.S.) 2, 3, 10. VI–IX.
- Adults can be swept from potatoes, in large numbers, any time during the summer.
- \* *Minettia* americana Mall. (G.S.) 10. V–VI.
- \*     *americanella* Shew. (G.S.) 10. V–VI.
- flaveola* Coq. 3. VI.
- \*     *lupulina* Fab. (C.C.) 3, 15, 18. VI–VII.
- \* *Homoneura* bispina Lw. (G.S.) 3. VII.
- \*     *lyraformis* Shew. (G.S.) 10. VI.
- \*     *seticauda* Mall. (C.C.) 3. VII.
- \* *Sapromyza* cyclops Mel. (C.C.) 3. VI–VIII.
- fusca* Shew. (G.S.) 10.
- \*     *hyalinata* Mg. (G.S.) 10. V–VIII.
- monticola* Mel. (G.S.) 18. VII, IX.
- \*     *ouelleti* Shew. (G.S.) 10, 12. V–VIII.

## DROSOPHILIDAE. Pomace flies

- \* *Drosophila* buscki Coq. (C.C.) 3, 10. II and X.
- \*     *funebis* Fab. (G.S.) 10, 15. IV–VI.

## ASTEIIDAE.

- \* *Astela* nr. *beata* Ald. (F.M.) 10. VI.  
Shewell states that this species is undescribed.

## AGROMYZIDAE.

- \* *Cerodontha dorsalis* Lw. (C.C.) 2. VI.
- \* *Agromyza angulata* Lw. (G.S.) 10, 20. VII.
- \* *artemisiae* Kalt. (C.C.) 2. V.
- \* *laterella* Zett. (G.S.) 10. VIII.
- \* *neptsi* Lw. (G.S.) 10. VI.

## PHYLLOMYZIDAE.

- \* *Hypaspistomyia halteralis* coq. (G.S.) 3. V-VII.
- \* *Phloeomyia indecora* Lw. (C.C.) 2, 3. V-VI.

## CHLOROPIDAE, (OSCINIDAE). Frit flies and stem maggots

- \* *Meromyza americana* Fit. (C.S.) 3, 18. VI-VII.  
Adults common in southern Alberta. No records of damage to wheat.
- \* *lineola* Curr. (E.S.) 15.  
*marginata* Beck. 15. VIII.
- \* *Chloropisca glabra* Mg. (C.S.) 3, 5, 8, 10. V-VII.  
Puparia taken in very large numbers from wheat stubble at Drumheller.  
Predator on root aphids.
- \* *grata* Lw. (C.S.) 3, 8. VII.
- \* *pulla* Lw. (C.S.) 3, 8. V-VI.
- \* *variceps* Lw. (C.S.) 8, 10, 12. IV-V and VIII-IX.  
Adults swarm on outside walls and windows. Often enter houses. Most abundant in autumn.
- \* *Epichlorops exilis* Coq. (C.S.) 10. VII.  
This large species is taken freely in slough grass.
- Chlorops ingrata* Curr. 3.
- \* *producta* Lw. (C.C.) 2. VI.
- \* *sulphurea* Lw. (C.C.) 2. VI.
- Gaurax festivus* Lw. 3. VII.  
Bred from a circular leaf mine in cottonwood poplar at Lethbridge.
- Madiza cinerea* Lw. 3, 5. V-VII.
- \* *oscinina* Fall. (C.S.) 15. VI.  
*parva* Adam. "Alberta".
- \* *Oscinella coxendix* Fit. (C.S.) 2, 3. VI.
- \* *pullicornis* Sab. (C.S.) 3, 10. V-VII.
- \* *frit* L. (C.S.) 3, 8. VI.  
Not common. No damage to grain noted.

## EPHYDRIDAE. Brine flies

- Psilopa compta* Mg. 3. V.
- \* *Ochthera mantis* deG. (E.S.) 2, 10. IV-VI and X.
- \* *Parydra bituberculata* Lw. (C.C.) 2, 10. VI and IX.
- \* *Notiphila scalaris* Lw. (C.C.) 3, 10. VI.
- Philygra fuscicornis* Lw. 3. V-VI.

This record from Canadian Entomological Record, 1916. The genus is not included in Curran's Diptera of North America.

**BORBORIDAE.** Small dung flies

- \* **Sphaerocera** subsultans Fab. (C.C.) 5, 10, 15, 18. V-VII.  
Larvae and adults very abundant in soil of well-manured seed bed of cabbages at Edmonton. No damage.
- \* **Leptocera** atra Adam. (C.C.) 3, 10. VI-VIII.
- \* fontinalis Fall. (C.C.) 2, 3. IV-VI.
- \* limosa Fall. (C.C.) 2, 10, 20. IV-VII.
- \* lutosa Stn. (C.C.) 2, 10, 20. VI-VII.
- \* **Borborus** equinus Fall. (C.C.) 3, 10, 20. V-VII.  
Adults very common, frequently almost cover fresh horse droppings.

**CLUSIIDAE.**

- \* **Clusiodes** melanostoma Lw. (E.S.) 10, 15. VI.

**CHAMAEMYIDAE.**

- \* **Leucopsis** americana Mall. (E.S.) 10. IX.  
Swept from potatoes heavily infested with Psyllids and a few aphids, at Edmonton. Larvae recorded as predators on aphids.
- \* **Plunomia** elegans Hall. (G.S.) 10. VI.

**TETANOCERIDAE (SCIOMYZIDAE).** Marsh flies

- \* **Sciomyza** simplex Fall. (E.S.) 8, 10. VI-VII.
- Pteromicra** canadensis Curr. 19. V.
- \* **Melina** albocostata Fall. (C.C.) 2. VI.
- \* nana Fall. (E.S.) 10. VI-VIII.
- \* obtusa Fall. (C.C.) 3. VIII.
- \* schoenherri Fall. (E.S.) 10. VII.
- \* vitalis similis Cress. (E.S.) 10. VIII.
- \* **Sepedon** armipes Lw. (E.S.) 8, 10. VI and IX.
- \* fuscipennis Lw. (C.C.) 3. VIII.
- \* pacifica Cress. (E.S.) 3. VIII.
- \* pusillus Lw. (E.S.) 10, 12, 15. VI and IX.
- \* **Dictya** umbroides Curr. (E.S.) 9, 19, 20. VII.
- \* **Hedroneura** lineata (? = rufa) (E.S.) 10, 15. VI-VIII.
- \* **Tetanocera** papillifera Mel. (C.C.) 2. VIII.
- \* phyllophora Mel. (E.S.) 20. VII.
- \* plebeia Lw. (C.C.) 20. VII.
- \* rotundicornis Lw. (E.S.) 10. VI.
- \* silvatica Mg. (E.S.) 8. VI.
- \* triangularis Lw. (C.C.) 3, 10. VIII.
- \* valida Lw. (E.S.) 7, 10. VI.
- \* vicina Macq. (C.C.) 3, 10, 20. VI-VIII.
- \* **Limnia** saratogensis Fit. (E.S.) 8, 9. VI.
- \* ottawensis Mel. (E.S.) 10. VII.

**CHYROMYIDAE.**

- \* **Trixoscelis** fumipennis Hall. (G.S.) 10, 12. VI.

**PSILIDAE.**

- \* **Pseudopsila** perpolita Joh. ? (G.S.) 12. IX.
- \* **Psila** atrata Mel. ? (F.M.) 15. VI.

**HELOMYZIDAE.**

- \* **Suilla** nemorum Mg. (C.C.) 10, 15. VI-VII.
- \* **Pseudoleria** vulgaris Garr. (E.S.) 3. VI.

## HELOMYZIDAE—concluded.

"*Leria*" pectinata Lw. "Alberta".

- \* *Helomyza* serrata L. (C.C.) 2, 3, V and VII.
- \* *Tephrochlamys* canescens Mg. (C.C.) 15, 18, V-VI.
- \* *Eccoptomera* americana Darl. (E.S.) 8, VI.
- \* *Anorostoma* marginata Lw. (F.M.) 15, VII.
- \* *Acantholeria* oedimius Garr. (C.C.) 10, VII-VIII.
- \* *Barbastoma* barbatus Garr. 19.

Recorded by Garrett, Ins. Ins. Mens. 1924. Genus not included in Curran.

## MUSCIDAE. Dung flies, House flies, Root maggots, etc.

## CORDYLURINAE or SCATOPHAGINAE Dung flies

*Allomyella* robusta Curr. 19, VII.

- \* *Scatophaga* (? = *Scopeuma*) furcata Say. (C.C.) 2, 19, V.
- \* *mordax* Fab. (C.J.) 6, VIII.

Adults recorded as feeding on leaf-hoppers. (Childs.)  
palpalis Mall. 19.

- \* *stercoraria* L. (F.S.) 2, 3, 6, 8, 10, 20, V-VII.
- \* *suilla* Fall. (F.S.) 10, 20, V-VII.
- \* *Acicephala* intermedia Curr. (C.C.) 1, VI.
- alberta Curr. 19, V-VII.
- \* *Cordilura* alberta Curr. (F.S.) 8, 19, VI-IX.
- \* *beringensis* Mall. (F.S.) 8, VI.
- \* *browni* Curr. (F.S.) 8, VI.
- \* *confusa* Lw. (F.S.) 8, 10, 12, V-VII.

Adults swarm among rushes by water.

- \* *fasciventris fulvithorax* Curr. (C.C.) 8, 19, VI-VIII.
- \* *latifrons* Lw. (F.S.) 8, 12, VI.
- \* *masconina* Curr. (F.S.) 8, VI.
- varicornis Curr. 19, V-VII.

- \* *Spaziphora* cincta Lw. (F.S.) 8, VI.

Adults abundant among rushes by water.

- \* *Pogonota* fulvibarbra Lw. (F.S.) 10, VII.

*Trichopalpus* nigribasis Curr. 19, VII-VIII.

- \* *Achaetella* varipes Wlk. (F.S.) 5, 8, 10, VI-VIII.

- \* *Parallelomma* pleuritica Lw. (F.S.) 8, 20, VI-VII.

Adults common on foliage near water.

- \* *Chaetosa* punctipes Mg. (F.S.) 8, 10, VI-VIII.
- \* *Hexamitocera* vittata Coq. (F.S.) 10, VI.
- \* *Orthacheta* brunneipennis Joh. (F.S.) 8, 12, 19, VI-VII.
- \* *hirtipes* Joh. (F.S.) 10, VIII.

*Amaurosoma* alberta Curr. 18, VI.

## MUSCINAE House fly sub-family

We have removed these from the Anthomyiinae, with which they are interspersed in Curran's key.

- \* *Musca* domestica L. (H.H.) 1-10, 12, 14, 18-21, I-XII.
- \* *Orthellia* (= *Cryptolucilia*) caesarion Mg. (R.S.) 1-3, 10, IV-VIII.
- \* *Pyrellia* cyanicolor Zett. (? = *serena*) (R.S.) 10, V-VI.
- \* *Morellia* micans Macq. (R.S.) 3, 10, VI-VIII.
- \* *Hypodermodes* solitaria Knab (J.A.) 6, 10, VI.
- \* *Mesembrina* latrielle R-D. (H.H.) 10, VII.
- \* *Graphomyia* maculata Scop. (R.S.) 8, 10, 20, VI-VII.

MUSCIDAE—*continued*.MUSCINAE—*concluded*.

- \* **Muscina** assimilis Fall. (R.S.) 10, 19. III-V and VIII-IX.  
Larvae recorded as mining in roots.
- \* **stabulans** Fall. (H.H.) 3, 6, 10. V-VIII.  
Larvae found in decaying vegetation, as parasitoids of other insects, and in human alimentary canal.
- Stomoxys** calcitrans L. 19.  
Recorded from Banff, but has not been found anywhere further north.
- \* **Haematobia** (**Lyperosia**) irritans L. (H.H.) 10. VIII.  
Appears to be rare in Alberta. No reports of attacks on cattle have been received.

## ANTHOMYINAE S.L. Root maggots, Leaf miners, etc.

Species belonging to this sub-family have been collected intensively in the southern portion of the province by Mr. H. L. Seamans. During the past three years we have made large collections in the northern part of Alberta. All collections have been determined by Dr. H. C. Hockett. Since nearly 30% of the species captured in 1937 were new records for the province, it is probable that the fauna in this sub-family is still very imperfectly known.

- \* **Eremomyoides** cylindrica Stn. (H.H.) 10, 12. V.
- \* **parkeri** Mall. (= *setosa*) (H.H.) 3, 10. IV-V.
- Hydrophoria** bispinosa Zett. 12. VII.
- \* **brunneifrons** Zett. (H.H.) 3, 10. V-VII.
- \* **divisa** Meig. (H.H.) 3, 10, 19. V-VIII.
- \* **flavohalterata** Mall. (H.H.) 10. VI.
- occulta** Meig. 12. VII.
- \* **ruralis** Meig. (H.H.) 19. IX.
- \* **subpellucens** Mall. (H.H.) 8. VI.
- \* **wierzejekii** Mik. (H.H.) 10. VI-VIII.
- \* **zetterstedti** Ring. (H.H.) 8, 10, 18. V-VII.
- \* **Calythea** micropteryx Thom. (H.H.) 10. X.
- \* **separata** Mall. (H.H.) 3. VIII.
- \* **Anthomyella** pratincta Panz. (H.H.) 3, 4, 10. V-VIII.
- \* **Pegomya** apicalis Stn. (H.H.) 18. VII.
- \* **connexa** Stn. (H.H.) 10. VIII.
- \* **duplicata** Mall. (H.H.) 10. VII.
- \* **flavicans** Stn. (F.S.) 10. VII.
- \* **geniculata** Bouch. (H.H.) 8, 10. VI-VIII.
- \* **hyoscyami** Panz. (H.H.) 3. VIII.

Though the "beet and spinach leaf-miner" has been taken in southern Alberta it does not appear to be of any economic importance here.

- \* **incisiva** Stn. (H.H.) 3. VI.
- \* **lipsia** Walk. (H.H.) 10. VIII-IX.
- \* **luteola** Mall. (H.H.) 10. VII.
- \* **nigroscutellata** Stn. (H.H.) 10. V and VIII.
- \* **quadrifida** Mall. (H.H.) 18. VII.
- unguiculata** Mall. 19. VII.
- \* **vittigera** Zett. (H.H.) 10. VIII.
- \* **Hammomyia** personata Coll. (H.H.) 10. IV-V.
- \* **unilineata** Zett. (H.H.) 10. IV-V.
- \* **unistriata** Zett. (H.H.) 10. V.

MUSCIDAE—*continued*.ANTHOMYINAE—*continued*.

- \* *Egle bicaudata* Mall. (H.H.) 10. IV-V.
- \* *fuscogalterata* Mall. (H.H.) 10. IV-V.
- \* *longipalpis* Mall. (H.H.) 10. V.
- \* *muscaria* Fab. (H.H.) 10. IV-V.
- \* *salicola* Huck. (H.H.) IV-V.

All species in this genus, which we have seen, visit salix blossoms in April and May, but have never been seen later in the year.

- \* *Paregle cinerella* Fall. (H.H.) 1-3, 6, 10, 18, 20. VI-VIII.
- \* *radicum* L. (H.H.) 3, 10, 15. V-X.
- \* *Prosalphia angustitarsis* Mall. (H.H.) 10. VI.
- \* *longipennis* Ring. (H.H.) 10, V.
- \* *silvestris* Zett. (H.H.) 10. VI-VIII.
- \* *Eremomyia incompleta* Stn. (H.H.) 2. III.
- \* *Hylemyia angusta* Stn. (H.H.) 1, 8, 10, 18. VI-VII.
- \* *antiqua* Mg. (H.H.) 2, 3, 10, 15. IV-VIII.

The onion maggot is widespread in the province.  
*aquitima* Huck. 18. VI-VII.

- \* *betarum* Lint. (H.H.) 10. IX.

Larvae mine in beet leaves. Of no economic importance in Alberta.

- \* *brassicae* Bouch. (H.H.) 3, 10, 15. V-VII.

The cabbage root-maggot is a serious pest in Alberta.  
*brevipalpis* Huck. 18. VII.  
*brunetta* Huck. 19. VII.

- \* *canadensis* Huck. (H.H.) 10, 19. V-VI.

Adults sometimes very abundant in brome grass. No larvae found.

- \* *cerealis* Gill. (H.H.) 1, 3. VI-VIII.

Larvae infest wheat in Montana. All larvae bred from this crop in Alberta have belonged to other species.

- \* *cilicrura* Zett. (H.H.) 1-4, 8, 10, 20. V-VIII.

Adults very abundant and widespread throughout province. No records of injury to wheat or corn have been received, though they are reported from Saskatchewan.

- \* *coenosiaeformis* Stn. (H.H.) 3, 10, 12, 20. V-VIII.
- \* *collini* Ring. (H.H.) 10. VI.
- \* *cruciferae* Huck. 3, 19. VI-VIII.
- \* *depressa* Stn. (H.H.) 3, 10. VI-VIII.
- \* *equifrons* Huck. 19. VII.
- \* *extremitata* Mall. (H.H.) 10. VII.
- \* *floralis* Fall. (H.H.) 3, 10. VI-VII.

In 1929 this fly destroyed large areas of stinkweed (*T. arvense*) in the Peace River District. The larvae inhabited the crowns of the plants which, in many instances, were entirely severed from the roots.

- \* *frontulenta* Huck. 3. V.
- \* *garretti* Huck. 20. VII.
- \* *gemina* Huck. 19. V-VI.
- \* *hinei* Mall. (H.H.) 10. IV-VII.
- \* *impersonata* Huck. (H.H.) 10. VI.
- \* *inornata* Stn. (H.H.) 15. VI.
- \* *lasciva* Zett. (H.H.) 8, 10. V-VII.
- \* *latipennis* Zett. (H.H.) 10. V-VI.
- \* *linearis* Stn. (H.H.) 10. IV-VI.
- \* *lineariventris* Zett. (H.H.) (= *uniseriata*) 8, 10, 18, 19. V-VIII.

MUSCIDAE—*continued*.ANTHOMYINAE—*continued*.**Hylemyia** lobata Huck. 19. V.

marginata Stn. 19. VII.

- \* montana Mall. (H.H.) 3. VII–VIII.

mutans Huck. 3. V.

- \* neomexicana Mall. (H.H.) 3, 6, 8, 10, 19, 20. VI–IX.

The larvae of this species, which have a black stigmal plate, have been bred from wheat seedlings at Granum.

nigricaudata Huck. 18. VII.

- \* normalis Mall. (H.H.) 3, 6. VI–VII.

Larvae bred from wheat seedlings at Granum.

- \* occidentalis Mall. (H.H.) 6, 10. V–VI.

pedicellaris Huck. 19. V.

- \* penicillaris Stn. (H.H.) 10. V.

pentaformis Huck. 18, 19, 20. VI–IX.

- \* pilifemur Ring. (H.H.) 10. V–VI.

- \* planipalpis Stn. (H.H.) 10, 19. V.

- \* pluvialis Mall. (H.H.) 3, 8, 10, 12, 19, 20. VI–VIII.

propinqua Huck. 18, 19. VII–IX.

- \* pullula Zett. (H.H.) 10. V.

repleta Huck. 19, 20. IV–VI.

- \* replicata Huck. (H.H.) 10, 20. V–VIII.

seamansi Huck. 18. VI.

- \* sepia Meig. (C.C.) 1. VI.

- \* setifer Mall. (H.H.) 10, 15, 20. VI–IX.

- \* setigera Joh. (H.H.) 1. VII.

- \* spiniventris Coq. (H.H.) 20. VII.

- \* tarsata Ring. (H.H.) 10, 15. V.

- \* testacea Stn. (H.H.) 3. VII–VIII.

- \* trilineata Stn. (H.H.) 20. VII.

- \* variata Fall. (H.H.) 10, 12. VI.

- \* **Lispe** antennata Ald. (H.H.) 10. VIII–IX.

palposa Wlk. (H.H.) 12, 20. VII–VIII.

- \* polita Coq. (H.H.) 3. VI.

- \* salina Ald. (H.H.) 2, 10. II, V and VIII.

- \* tentaculata deG. (H.H.) 10. VIII.

- \* uliginosa Fall. (H.H.) 3, 10, 12, 19. VI–VIII.

- \* **Pseudophaonia** orichalcea Stn. (H.H.) 10, 20. V–VIII.

**Hoplogaster** flavidipalpis Huck. 19. VI.

- \* gilva Zett. (H.H.) 10. VI–VII.

- \* mōllicula Fall. (H.H.) 12, 18, 20. VII–VIII.

octopuncta Zett. 19. VII.

- \* **Schoenomyza** chrysostoma Lw. (C.C.) 1, 10. V–VI.

- \* dorsalis Lw. (H.H.) 2, 3, 8, 10, 19. V–VIII.

partita Mall. 19. VI.

- \* sulfuriceps Mall. (H.H.) 10, 19. VI and IX.

- \* litorella Fall. (H.H.) 3, 20. VII–VIII.

- \* **Coenosia** aliena Mall. (H.H.) 18. VI.

alticola Mall. 3. VI.

- \* argenticeps Mall. (H.H.) 3, 10. VI.

- \* compressa conforma Huck. (H.H.) 10, 20. VII–VIII.

- \* incisurata V.d.W. (H.H.) 10. VI.

lata Walk. 1. V.

MUSCIDAE—*continued*.ANTHOMYINAE—*continued*.

- \* **Coenosia** nigrescens Stn. (H.H.) 12. VI.
- \*   pallipes Stn. (H.H.) 3, 10, 19, 20. VI–VIII.
- \* **Limosia** anthracina Mall. (H.H.) 18–20. VII.
- \*   cilicauda Mall. (H.H.) 2, 3, 8, 10, 18. V–IX.
- \*   johnsoni Mall. (H.H.) 10, 18, 19, 21. VII–VIII.
- nigricoxa Stn. 19. VI.
- \*   pedella Fall. (H.H.) 8, 10, 19. VI–VII.
- pygmaea Zett. 19. VI.
- \*   triseta Stn. (H.H.) 3, 20. VI–VII.
- Pseudocoenosia** brevicauda Huck. 21. VII.
- longicauda Zett. 19. VI–IX.
- \* **Lispocephala** alma Meig. (H.H.) 10. V.
- pallipalpis Zett. 3, 10. IV–VII.
- \*   erythroceras Desv. (H.H.) 2, 3, 10, 20. V–X.
- \*   rubicornis Zett. (F.S.) 20. VII.
- \* **Macrorchis** alone Walk. (H.H.) 10. V.
- \* **Limnospila** albifrons Zett. (H.H.) 10. VI–VIII.
- \* **Azelia** gibbera Meig. (H.H.) 20. VII.
- \*   triquetra Zett. (H.H.) 20. VII.
- \* **Fannia** canicularis L. (H.H.) 3, 10, 19. V–IX.
- \*   carbonaria Meig. (H.H.) 10. VI.
- \*   genualis Stn. (H.H.) 10. VIII.
- \*   glauescens Zett. (H.H.) 3. V–VII.
- \*   immaculata Mall. (H.H.) 10. VI.
- \*   incisurata Zett. (H.H.) 3. VI.
- \*   laevis Stn. (H.H.) 10. VI.
- \*   manicata Meig. (H.H.) 10. VIII.
- \*   pallidiventris Mall. (H.H.) 12. VII.
- \*   postica Stn. (H.H.) 10. VI.
- \*   scalaris Fab. (H.H.) 3, 10, 15, 19. V–IX.
- \*   serena Meig. (H.H.) 10. VI.
- \*   sociella Zett. (H.H.) 10. V.
- \*   spathiophora Mall. (H.H.) 10. VII.
- tibialis Mall. 3, 18. V–VII.
- \* **Limnophora** discreta Stn. (H.H.) 10, 19, 20. VI–VII.
- narona Walk. 20. VIII.
- (**Pseudolimnophora**) nigripes R-D. 20. VII.
- (**Lispoides**) aequifrons Stn. 6. IX.
- (**Sphenomyia**) biquadrata Walk. 19.
- (**Spilogona**) alberta Huck. 19. VI–VII.
- alliterata Huck. 19. VII.
- alticola Mall. 19. VI.
- anthrax Big. 18, 19. VII.
- argentiventris Mall. (H.H.) 3. VI.
- occidentalis Huck. 19. VIII.
- brevicornis Mall. 3. VI.
- concolor Stn. (H.H.) 10, 19–21. VII–VIII.
- cretans Huck. 3. V–VI.
- fimbriata Huck. 18. VII.
- fumipennis Zett. (H.H.) 10. VI.
- fuscomarginata Huck. 19. VII.
- gibsoni Mall. (H.H.) 10, 18. VI–VII.

MUSCIDAE—*continued*.ANTHOMYINAE—*continued*.**Limnophora** *imitatrix* Mall. 19. VI–VII.

- \* *leucogaster* Zett. (H.H.) 10. V–VIII.
- \* *magnipunctata* Mall. 18. VI–VIII.
- \* *narina* Walk. (H.H.) 10. VII.
- \* *rufitarsis* Stn. 18, 21. VII–VIII.
- \* *sectata* Huck. 19. VII.
- \* *subrostrata* Stn. 19. VII.
- \* *surda* Zett. 19, 20. VI–VII.
- \* *suspecta* Mall. (H.H.) 10. VI.
- \* *tetrachaeta* Mall. (H.H.) 3. VI–VIII.
- \* *trigonifera* Zett. (H.H.) 10. VII–VIII.
- \* **Myospila** *meditibunda* Fab. (H.H.) 3, 10, 18. V–VIII.
- \* **Mydaea** *brevipilosa* Mall. (H.H.) 10. VI.
- \* *discimana* Mall. (H.H.) 10, 20. V–VII.
- \* *occidentalis* Mall. (H.H.) 10. VI–VIII.
- \* *persimilis* Mall. (H.H.) 10, 19. VI.
- \* *punctata* Stn. 3. VI.
- \* **Lasiopt** *conformis* Mall. (H.H.) 19, 20. VII.
- \* *innocuus* Zett. (H.H.) 10, 19, 20. VI–VII.
- \* *johnsoni* Mall. (H.H.) 10, 12. VI.
- \* *latipennis* Mall. (H.H.) 10. VII.
- \* *melanderi* Mall. (H.H.) 10. V–VI.
- \* *septentrionalis* Stn. (H.H.) 15, 18–20. VII–IX.
- \* *spiniger* Stn. (H.H.) 10, 20. VI–VII.
- \* **Alloestylus** *diaphanus* Wied. (H.H.) 10. VI and IX.
- \* **Bigotomyia** *houghi* Stn. (H.H.) 10. VII.
- \* **Dendrophæonia** *querceti* Bouch. (H.H.) 10. VIII.
- \* **Ophyra** *leucostoma* Weid. (H.H.) 3, 8, 10, 18. VII–VIII.
- \* **Spilaria** *lucorum* Fall. (H.H.) 8, 10, 18. VI–VIII.
- \* *marmorata* Zett. (H.H.) 10, 20. VI–VII.
- \* *punctata* Stn. (H.H.) 2, 3, 10, 18. V–VII.

Several bred from puparia taken from soil under red currant bushes, at Edmonton.

- \* **Helina** *barpana* V.d.W. (H.H.) 10, 15. VI–IX.
- \* *brevis* Mall. (H.H.) 1, 3, 10, 20. VI–VIII.
- \* *duplicata* Mg. (H.H.) 1–3, 6, 10, 20. V–VIII.
- \* *flavocalyptata* Stn. (H.H.) 10, 18, 20. VI–VII.
- \* *fulvisquama* Zett. (H.H.) 18. VII.
- \* *lafifrontata* Mall. (H.H.) 10, 19. VII–VIII.
- \* *marmorata* Zett. (H.H.) 10. VI–VIII.
- \* *multiseriata* Mall. (H.H.) 3. VII–VIII.
- \* *neopociloptera* Mall. (H.H.) 10. IV–V.
- \* *nigricans* Mall. (H.H.) 6, 10, 20. V–VIII.
- \* *nigripennis* Walk. (H.H.) 8, 10. VI–VIII.
- \* *nigrita* Mall. (H.H.) 10. VII.
- \* *nitida* Stn. (H.H.) 10. VIII.
- \* *obscurata* Mg. (H.H.) (= *nasoni*) 10. VI–VIII.
- \* *pectinata* Joh. (H.H.) 10. VI.
- \* *procedens* Walk. (H.H.) 10. VII.
- \* *rufitibia* Stn. (H.H.) 10. VI.
- \* *tuberculata* Mall. 19. VII.

## MUSCIDAE—concluded.

## ANTHOMYINAE—concluded.

- \* **Phaonia** *apicata* Joh. (H.H.) 8, 10. VI.
- \* *basiseta* Mall. (H.H.) 3, 6, 8, 10, 18. V-VI.
- \* *brunneinervis* Stn. (H.H.) 10, 18. VI-VII.
- \* *bysia* Walk. (H.H.) 10. VII-VIII.
- \* *caerulescens* Stn. (H.H.) 10. V.
- \* *consobrina* Zett. (H.H.) 10. V.
- \* *errans* Mg. (H.H.) 10. VIII.
- \* *frenata* Holm. (H.H.) 10. IX.
- harti* Mall. 8.
- \* *monticolor* Mall. (H.H.) 18-20. VII.
- \* *nigricans* Joh. (H.H.) 10. V.
- pallida* Stn. 18. VII.
- \* *protuberans* Mall. (H.H.) 19. VIII.
- \* *rufibasis* Mall. (H.H.) 3. VII.
- \* *serva* Meig. (H.H.) 10, 12. VI-VIII.
- \* *soccata* Walk. (H.H.) 10. VI.
- \* *solitaria* Stn. (H.H.) 10. VI
- trivialis* Mall. 19.
- \* **Hydrotaea** *acuta* Stn. (H.H.) 3. VI.
- \* *armipes* Fall. (H.H.) 3, 10. V-VII.
- \* *cristata* Mall. (H.H.) 12, 18. VI-VII.
- \* *dentipes* Fab. (H.H.) 15. VI.
- \* *houghi* Mall. (H.H.) 18, 20. VI-VII.
- \* *meteorica* L. (H.H.) 3, 7, 8, 10, 18, 20. VI-VIII.
- The adults are the most persistent feeders on perspiration, in Alberta. Most abundant in shady places.
- \* *militaris* Meig. (H.H.) 10. VII-IX.
- \* *occulta* Meig. (H.H.) 10. VIII.
- \* *palaestrica* Meig. (H.H.) 10. V.
- \* *scamba* Zett. (H.H.) 10, 12, 20. VI-VIII.
- \* *unispinosa* Stn. (H.H.) 19, 20. VII.
- \* **Hebecnema** *affinis* Mall. (H.H.) 10. VI.
- \* *vespertina* Fall. (H.H.) 10, 12. VI-VIII.
- \* **Phyllogaster** *littoralis* Mall. (H.H.) 2, 3, 8. VII-IX.
- unicus* Stn. 3. IX.
- Pogonomyia** *aldrichi* Mall. 19. VII.
- errans* Meig. 18. VII.
- latifrons* Mall. 18. VI.
- \* *minor* Mall. (H.H.) 1, 18. VI.
- \* *nitens* Stn. (H.H.) 10, 18, 20. VI-VIII.
- \* *similis* Mall. (H.H.) 10, 18-20. VI-VII.

## GASTEROPHILIDAE. Bot flies of horses

- \* **Gasterophilus** *intestinalis* deG. 2, 3, 10. VII-IX.
- haemorrhoidalis* L. "Alberta".
- \* *veterinus* Clk. 3, 6, 10. IX.

## METOPIIDAE. Flesh flies and blow flies

- \* **Taxigramma** *heteroneura* Mg. (C.C.) 2. VII.
- Hilarella** *fulvicornis* Coq. 3. VII.
- \* **Metopia** *leucocephala* Rossi. (C.C.) 2, 10. VI-VII.

Taken in numbers, on sandy bank inhabited by various bees and wasps.

## METOPIIDAE—concluded.

- \* **Wohlfahrtia** meigenii Sch. (F.M.) 3, 10. VIII.  
Bred from foot of tame rat. Adults on goldenrod and sweet clover.
- \* **Senotainia** trilineata V. deW. 3. VII.
- \* **Cynomya** cadaverina R.D. (R.S.) 2, 3, 6, 10. IV–IX.
- \* **Calliphora** elongata Hgh. 3. VII.  
erythrocephala Meig. 3. X.
- \* latifrons Hgh. (R.S.) 3. VI.  
montana Shn. 10. VIII.
- \* vomitoria L. (R.S.) 3, 6, 10, 18. V–X.
- \* **Phormia** regina Mg. (R.S.) 2, 3, 6, 10. II–VIII.
- \* terra-novae R.D. (R.S.) 2, 3, 6, 10. II–V.
- \* **Lucilia** caesar L. (R.S.) 2, 10. V–VI.
- \* sericata Mg. (R.S.) 3. V–VIII.
- \* sylvarum Mg. (R.S.) 3, 10. VII–VIII.
- \* **Pollenia** rudis Fall. (F.M.) 10. III–X.  
This earthworm parasite was first taken in Alberta at Edmonton in 1935.  
Was very abundant in 1936 and 1937.
- \* **Sarcophaga** aculeata Ald. (C.C.) 1. VII.
- \* aldrichi Park. (F.M.) 10. VI.  
Recorded as parasite of tent caterpillar, (Caesar).
- \* allantis Ald. (F.M.) 4. VI.
- \* bisetosa Park. (F.M.) 10. VI.  
canadensis Hall. 19. VI.
- \* cimbicis Twms. (F.M.) 8, 10. VI–VII.
- \* cooleyi Park. (C.C.) 3, 10. VIII.
- \* falciformis Ald. (F.M.) 3, 20. VII–VIII.  
Grasshopper parasite.
- \* harpax Pand. (C.C.) 18. VII.
- \* hunteri Hgh. (E.S.) 3. VII.  
Grasshopper parasite.
- \* kellyi Ald. (F.M.) 3. VI.  
Grasshopper parasite.
- \* latisterna Park. (F.M.) 10. VII–VIII.  
Recorded as cabbage butterfly parasite.
- \* l'herminieri R.D. (E.S.) 1, 3, 10, 18. VI–VIII.
- \* opifera Coq. (E.S.) 3. VI.
- \* pachyprocta Park. (F.M.) 7 sandy. VI.
- \* peniculata Park. (F.M.) 10, 20. VI–VII.
- \* scoparia Pand. (C.C.) 10. VII–VIII.
- \* sinuata Meig. (F.M.) 10. VII.  
Recorded as grasshopper parasite.
- \* **Agria** affinis Fall. (F.M.) 10. VII.  
Recorded as parasite of *V. antiopa*. (Anderson).

## CUTEREBRIDAE. Robust bot flies

- \* **Cuterebra** grisea Coq. (C.C.) 10, 17. VI–VII.  
Several bots in mice have been forwarded to us for determination, but all have been dead on arrival.

## OESTRIDAE. Bot flies of sheep and cattle

- \* **Oestrus** ovis L. 4, 10. VI.  
Larvae have been taken in numbers from heads of sheep raised near Edmonton.
- \* **Hypoderma** bovis L. (Hadwen) 3, 8. VII.

## TACHINIDAE. Parasitoid flies

- \* **Hystieria** abrupta Wied. (C.C.) 10, 18. VII-VIII.
- \* **Cylindromyia** compressa Ald. 19.
- \* **dosiades** Walk. (C.C.) 8, 10, 19. VI-VIII.
- euchenor Walk. 2. VII.
- \* **intermedia** Meig. (C.C.) 3, 10, 18. VI-VIII.
- \* **Epalpus** signifera Walk. (C.C.) 8, 10. IV-V.
- Archytas** lateralis Mcq. 2. VIII.
- \* **Cnephallodes** algens Wd. (C.C.) 6, 8, 10, 18. VII.
- ampliforceps Row. 6, 8. VI-VII.
- \* **argentea** Row. (C.C.) 15, 19, 20. VII-IX.
- canadensis Toth. 18, 20. VII.
- \* **emarginata** Toth. (C.C.) 10. VI.
- \* **hispidata** Toth. 10, 18. VI-VIII.
- \* **latianulum** Toth. (C.C.) 10, 15. VII-VIII.
- latiforceps Toth. 19. VI.
- \* **latifrons** Toth. (C.C.) 1, 3, 10. VII-VIII.
- \* **piceifrons** Tns. (C.C.) 3, 6, 8, 10. V and VIII-IX.
- \* **pilosa** Toth. (C.C.) 6, 10, 19. VII-IX.
- \* **rostrata** Toth. (C.C.) 3, 10, 15, 19. VII-IX.
- \* **Belvosia** bifasciata Fab. (C.C.) 6. VII.
- canadensis Curr. 6. IX.
- \* **Parademoticus** piperi Coq. (C.C.) 10. VIII.
- Arctophyto** marginalis Toth. 19. VII-VIII.
- wickmani Tns. (C.C.) 18, 19. VII-VIII.
- \* **Cyrtophloeoba** horrida Coq. (C.C.) 18. V.
- Gymnophania** montana Coq. 3. V.
- \* **Eulasiana** comstocki Tns. (C.C.) 10. VI.
- \* **Myophasia** clistoides Tns. (C.C.) 10. VII.
- \* **Polidaria** aeros Walk. (C.C.) 10. VIII.
- \* **Linnaemyia** compta Fall. (C.C.) 3. V-VI and VIII.
- An important parasite of cutworms in southern Alberta.
- \* **haemorrhoidalis** Fab. (C.C.) 8, 10. VI-VIII.
- \* **Lypha** dubia Fall. ? (F.M.) 10. IV.
- \* **Mericia** ampelus Walk. (C.C.) 10. VIII.
- Recorded as parasite of fall webworm. (Tothill).
- \* **arcuata** Toth. (C.C.) 10. VI.
- Recorded as parasite of fall webworm. (Tothill).
- \* **flavicornis** Br. (C.C.) 10. VI.
- \* **nigripalpis** Toth. (C.C.) 4, 10. VI and VIII.
- \* **sulcocarina** Toth. (C.C.) 10, 20. VI-VII.
- \* **Rhynchodexia** confusa West. (C.C.) 8, 10. VII-VIII.
- \* **Imitomyia** sugens Lw. (C.C.) 10. VIII.
- \* **Melanophrys** insolita Walk. (C.C.) 3. VI.
- \* **Xanthomelana** arcuata Say (C.C.) 2. VI.
- \* **Besseria** brevipennis Lw. (C.C.) 2, 3. V-VI.
- \* **Gymnosoma** fuliginosa Des. (C.C.) 2, 3, 18. VI-VII.
- Recorded as a parasite of the wheat bug. (Knowlton).
- \* **occidentale** Curr. (C.C.) 10. V and VIII.
- Phasia** occidentalis Walk. 3, 18. VII.
- \* **Alophora** aenovenstris Will. (C.C.) 12. VIII.
- \* **fenestralis** Big. (C.C.) 12. VIII.
- \* **nitida** Coq. (C.C.) 12. VIII.
- \* **semiciherea** Mg. (C.C.) 2, 3, 10. VI-VII and IX.

TACHINIDAE—*continued*.

- \* (*Hyalomya*) *occidentis* Wlk. 2, 6. V and VIII.
- \* *Voria americana* V.d.W. (C.C.) 10. VI–VII.  
*rigidirostris* V.d.W. 3. VIII.
- \* *ruralis* Mg. (C.C.) 10. VII–VIII.
- \* (? = *Blepharigena*) *spinulosa* Big. (C.C.) 10, 18. VI–VII.
- \* *Cryptomeigenia dubia* Curr. ? (F.M.) 10. V–VII.
- \* *triangularis* Curr. (F.M.) 10. V–VI.
- \* *Steveniopsis sinuata* Tns. (C.C.) 2. VIII.
- \* *Clistomorpha alberta* Curr. 12. VI.
- \* *didyma* Lw. (C.C.) 10. VIII–IX.
- \* *triangulifera* Lw. (C.C.) 10, 12. VIII.
- \* *Winthemlia fumiferanae* Toth. (C.C.) 10. V.
- \* *quadripustulata* Fab. (C.C.) 8, 10, 20. VI–VIII.  
Parasite of many caterpillars, including Redbacked cutworm.
- \* *rufopicta* Big. (C.C.) 8, 10. VI.
- \* *sinuata* Rein. (C.C.) 10. VIII.
- \* *Merlana septentrionalis* Curr. 2. IV.
- \* *Pateloa silvatica* A. & W. (C.C.) 10. V–VII.
- \* *Doryphorophaga doryphorae* Ry. (C.C.) Bred VIII.  
Bred from potato beetle larvae at Edmonton, 1936.
- \* *Phorocera claripennis* Macq. (C.C.) 3, 10. VI.
- \* *sternalis* Coq. (C.C.) 10. VI.
- \* *Metaphyto genalis* Coq. (C.C.) 10. V.
- \* *Ernestia flavicornis* Br. (C.C.) 10, 18. VI–VII.
- \* *frontalis* Toth. (C.C.) V and VIII.
- \* *longicarina* Toth. 18. VII.
- \* *truncata* Zett. ? (C.C.) 10. V–VI.
- \* *Nemorilla floralis* Fall. (C.C.) 3. V–VI.
- \* *Zenilla affinis* Fall. (C.C.) 10. VII.
- \* *blanda* O.S. (C.C.) 10. VIII.
- \* *caesar* Ald. (C.C.) 10. VIII.
- \* *chelopiae* Rand. (C.C.) 8, 10, 18. V–VII.
- \* *confinis* Fall. (C.C.) 12. VIII.
- \* *eudryae* Tns. (C.C.) 10. VIII.
- \* *futilis* O.S. (C.C.) 8, 10. VII.
- \* *reclinata* A. & W. 18. VII.
- \* *submissa* A. & W. 18. VIII.
- \* *valens* A. & W. (C.C.) 10, 20. VII–VIII.
- \* *vulgaris* Fall. (C.C.) 10. VIII–X.

Host records for nearly all of these species are given by Aldrich and Webber, Proc. U.S. Nat. Mus. vol. 63. 1924.

***Gonia aldrichi*** Toth. 3. IV.

Bred from Pale Western and Redbacked cutworms.

- \* *breviforceps* Toth. (C.C.) 2–4, 10, 19. V–VI.
- \* *brevipulvilli* Toth. (C.C.) 2. IV.
- \* *fissiforceps* Toth. (C.C.) 18–20. VII.
- \* *frontosa* Say (C.C.) 2, 10, 12. IV–VI.
- \* *longiforceps* Toth. (C.C.) 2, 3, 7. IV–VI.

An important parasite of Pale Western cutworms.

- \* *longipulvilli* Toth. (C.C.) 3, 10. IV–V.

TACHINIDAE—*continued*.

- Peleteria aenea** Stg. 6. VII.  
 alberta Curr. 19. VIII.
- \* **anaxias** Wlk. (C.C.) (? = apicalis) 4, 10, 18. VI–VIII.  
 angulata Curr. 19. VII–VIII.
  - \* **bryanti** Curr. (C.C.) 3, 10, 12. VII–VIII.
  - \* **campestris** Curr. (C.C.) 2, 3, 6, 10, 12, 15. VI–VIII.
  - \* **clara** Curr. (C.C.) 2, 3, 6. VI–IX.
  - \* **confusa** Curr. (C.C.) (? = apicalis) 10, 12. VII–IX.  
 cornuta Curr. 1, 6. VI–VII.  
 cornuticaudata Curr. 19. VIII–IX.
  - \* **eronis** Curr. (C.C.) 2–4, 6. VI and VIII–IX.
  - \* **iterans** Wlk. (C.C.) 2, 8, 10. V–VII.  
 phairi Curr. 19. VIII.  
 prompta Mg. 3. IX.
  - \* **Hesperodina** cinerea Tns. (C.C.) 6, 10. VII–VIII.
  - \* **Admontia** pollinosa Curr. (F.M.) 10. VII.
  - \* **Gibsonomyia** nigricosta Curr. (C.C.) 19, 20. VII.
  - Phyllomya alberta** Curr. 12. VII.
  - Wagneria cinerosa** Coq. 3. Bred.  
 distincta Curr. "Alberta".
  - \* **helymus** Walk. (C.C.) 6, 8, 10. V–VII.
  - \* **sequax** Will. (C.C.) 3, 19. V.  
 Bred from Army cutworm.
  - \* **Æstrophasia** clausa Br. (F.M.) 10. V.
  - \* **Sturmia** inquinata V. d. W. (C.C.) 6. Bred.
  - \* **phycioides** Coq. (C.C.) 10. VII.
  - \* **ricinorum** Tns. (C.C.) 6.
  - \* **Achaetoneura frenchii** Will. (C.C.) 10. Bred.  
 Parasite of Forest Tent caterpillar.  
 malacosomae Curr. 3. VIII.
  - \* **Exorista mella** Walk. (C.C.) 6, 8, 10, 18. VI–VIII.  
 Parasite of Forest Tent caterpillar.
  - \* **simulans** Mg. (C.C.) 1, 8, 10, 12. VII–VIII.
  - \* **Anachaetopsis** tortricis Coq. (C.C.) 10. IX.
  - \* **Elodia barbata** Coq. (C.C.) 10, 18. VI–VIII.
  - \* **Tachinomyia panaetius** Wlk. (C.C.) 3, 10. VI–VII and IX.
  - \* **variata** Curr. (C.C.) 3, 8, 10. V–VII.
  - \* **Allophocera montana** Sm. (C.C.) 2. IV.  
**Actia interrupta** Curr. 19.
  - \* **Siphona cristata** Fall. (C.C.) 10. VII.
  - \* **geniculata** deG. (C.C.) 10. VI.
  - \* **Aphria ocypterata** Tns. 10, 18, 20. VII.
  - \* **Pelatachina** limata Coq. (C.C.) 8. VI.
  - \* **Lixophaga alberta** Curr. (F.M.) 10. VII.  
 parva Tns. (C.C.) 10. VIII.
  - \* **variabilis** Coq. ? (C.C.) 10. VII.
  - \* **Laximasicera** sexualis Curr. (C.C.) 10, 12, 18. VI–VII.
  - \* **Lydella myoidea** Desv. (C.C.) 10. VII–VIII.
  - \* **senilis** Mg. (C.C.) 10, 12, 18. VII–VIII.
  - \* **Acemya tibialis** Coq. (C.C.) 6. VI.

TACHINIDAE—*concluded*.

The following species are recorded in genera which are not included in Curran's "North American Diptera".

**Apostrophus** anthophilus Lw. 10. VII.

\* **Euptilopareia** erucicola Coq. (C.C.) 20. VII.

**Heteropterina** nasoni Coq. 18. VIII.

**Pseudotachinomyla** Webberi Sm. 2. IV.

## HIPPOBOSCIDAE. Louse flies

\* **Ornithoctona** erythrocephala Lea. (J.B.) 12.

From Burrowing hawk.

\* **Ornithomyia** fringillina Curt. (J.B.) 10, 12. VIII.

From chickadee, several species of sparrow, shrike, grosbeak, woodpecker.

\* **Lynchia** botaweirorum Swenk (J.B.) 12.

From bittern.

\* **Melophagus** ovinus L. (E.S.) 8, 10. VII.

Common on sheep around Edmonton. Probably occurs throughout the province where sheep are raised.



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## A METHOD OF DETERMINING THE MEAN SPEED OF MOVEMENT OF INSECTS IN A MASS OF FLOUR<sup>1</sup>

BY JOHN STANLEY<sup>2</sup> AND B. N. SMALLMAN<sup>3</sup>

### Abstract

A method of determining the mean speed of movement of adults of the flour beetle *Tribolium confusum* Duv., moving through a mass of flour, is described. The method consists of allowing a beetle to wander for a suitable length of time through a mass of flour made up of alternate black and white layers of flour, each 1 mm. thick. The motion of the beetle's legs churns the two colours into one another as it tunnels through the flour. If the flour mass be compressed into a solid cake, and sectioned at right angles to the laminae, the course of the tunnel is marked by gray traces owing to this mixing. From a study of these traces in section, the trail may be reconstructed and the distance traversed in unit time computed.

### Introduction

In the course of certain investigations of the growth of populations of the flour beetle, *Tribolium confusum* Duv. (Stanley, 1-3), one of us (J.S.) required a value for the mean speed of movement of the adult beetles as they wandered at random through the flour. Following numerous unsuccessful attempts, the writers have developed a very satisfactory method based on an idea conceived by the senior author. Briefly, the method consists of laying down a laminated mass of flour consisting of alternate black and white layers, each 1 mm. thick. A beetle is allowed to bore through this mass for a suitable time, and during this journey, the motion of the beetle's legs churns the two colors into one another along the path, so that if the mass is later compressed into a hard block and sectioned at right angles to the laminae, traces of the trail can be seen as gray marks on the faces of the sections. From a study of these traces the trail can be reconstructed, and the distance travelled in unit time computed.

### Experimental Methods

The laminated flour mass is laid down in a heavy-walled steel mold (Fig. 1) made from a short length of seamless steel tubing. The removable bottom is held in place by six heavy machine screws with their heads countersunk into the under side of the bottom. The dimensions of the mold are: inside

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diameter, 3 in.\*; outside diameter, 4.5 in.; wall thickness, .75 in.; inside depth, 4 in.; thickness of bottom, .75 in.

When the flour mass is to be compressed, a solid steel piston (Fig. 1) is inserted in the top of the mold. The dimensions of the piston are: length, 4.75 in.; diameter for 4 in. of the length, 2.95 in., for the remainder, 3.25 in. A pressure of 100,000 lbs. is applied in a large press used at Queen's University for testing the strength of materials. As the total area of the top of the mass is 7.0686 sq. in., a pressure of approximately 14,128 lb. per sq. in. is exerted. This produces a hard chalk-like cake which is expressed from the mold, after removal of the bottom, by use of an arbor press. Both piston and mold are very accurately machined and are heavily nickel-plated over an undercoat of copper.

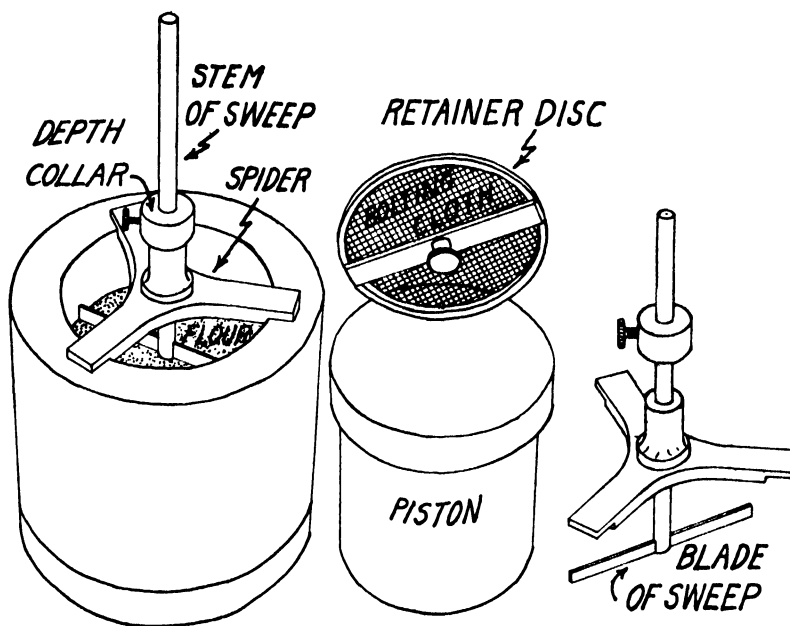


FIG. 1. Complete assembly of mold, spider and sweep; retainer disc; piston; sweep assembly.

The laminae are laid down as follows: a brass spider (Fig. 1) fits accurately into the top of the mold and holds a "sweep" with its stem (.25 in. diameter) accurately in the centre line of the mold. The sweep can be supported with the bottom edge of its blade at any height above the bottom of the mold by setting a small collar at the appropriate graduation mark on the stem. The 1-mm. graduations of the stem are not marked in Fig. 1 as they are too fine to show properly. Having set the sweep blade just 1 mm. above the bottom of the mold, a measured amount of white flour sufficient to make a layer

\*The mold was constructed with a diameter measurable in inches because it was at first intended to use laminae .05 in. thick. The change to metric measurements was made because of the ease of obtaining high grade 1-mm. co-ordinate paper.

1 mm. thick over the bottom is poured in and is then smoothed off by cautiously rotating the sweep to and fro. Some trouble has been experienced through tearing of the first lamina, owing to the small coefficient of friction between the flour and the smooth bottom of the mold. However, when the first lamina has been laid down the others follow easily, with successive 1-mm. elevations of the sweep blade for each layer. Small quantities of extra flour are removed with a small shovel shaped like a hoe. The black layers are made from flour into which 1.5% of purified lamp-black has been intimately mixed in a pebble mill. Altogether 91 layers are laid down, Nos. 1, 3, 5, . . . 91 being white, Nos. 2, 4, 6, . . . 90 being black.

The beetle is then placed on top of this flour mass and watched until it bores in, whereupon a retainer disc (Fig. 1), consisting of a brass ring covered with silk bolting cloth, is gently lowered onto the flour to prevent the beetle from coming out and wandering on top without boring.

After a suitable period, usually 100 hr., during which the assembly is held at constant temperature, compression is carried out as described above, following which the resultant cake is glued into a hardwood cradle to support it during the subsequent sectioning.

Section at right angles to the laminae is effected by routing off successive 1-mm. layers by means of a routing cutter rotating at 5,000 r.p.m. in a Delta Triple Duty drill press. It is interesting to note that the mass is very abrasive, and rapidly dulls the cutter. The senior author, who is now carrying on the work, is considering the use of a cutter tipped with stellite.

As each face is exposed, it is mapped on 1-mm. co-ordinate paper, and these maps are subsequently trans-illuminated in pairs and larger groups to see that the traces line up properly, and that no small parts have been missed. Occasionally a few millimetres are missed. These parts are inked in by hand, but as they are small and of obvious size and shape this does not result in any appreciable loss of accuracy. Unfortunately the sections cannot be reproduced by photography, because the downward movement of the upper laminae during compression produces a central "dishing" of the laminae in the middle of the block. In section therefore, the upper and lower laminae appear as straight lines, being in contact with parts of the mold during compression, while the middle layers are curved. It is necessary for purposes of computation to transpose the maps of the sections to co-ordinate paper having straight lines for all laminae, homologous with the original flat form of the laminae before compression.

These retouched maps are then traced with india ink on to 1-mm. sheets of "lumarith", a transparent celluloid-like material. The necessary 76 sheets of lumarith are previously cut about 3.1 in. by 93 mm., stacked up, and clamped together. The edges of the resultant block are carefully machined and polished to form a rectangular block 3 in. by 3 in. by 91 mm., *i.e.*, just the size to enclose the uncompressed flour mass within rectangular planes, with the component layers of lumarith at right angles to the black and white flour laminae. When the traces are marked on these prepared sheets,

and the sheets stacked, a transparent block results with a black replica of the beetle's trail suspended within its substance.

Two procedures are subsequently followed. If simply the length of the trail produced per unit time is desired, the co-ordinates of numerous points along its centre-line are found from a study of the traces in the dissectible lumarith block, and the sum of the distances between them computed from the familiar formula of co-ordinate geometry. From this the distance travelled in unit time is readily found.

If a solid model of the trail is desired, the traces are cut through each sheet by means of a small router, the sheets restacked, and melted Wood's metal is injected into the resultant worm-like cavity. The lumarith is then cut away, and the step-like annulations running around the model smoothed off with a high-speed dental burr. Owing to the waste of expensive lumarith, it is not planned to make many such models.

Owing to the extraordinarily laborious nature of the process, only one block has as yet been run through, but others are being worked on. When sufficient data has accumulated to compute a statistically sound value for the mean speed, this value will be reported.

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# AN ACCOUNT OF A PARASITIC COPEPOD, *SALMINCOLA SALVELINI* SP. NOV., INFECTING THE SPECKLED TROUT<sup>1</sup>

BY LAURENCE R. RICHARDSON<sup>2</sup>

## Abstract

An account and description are given of *Salmincola salvelini* sp. nov., a parasitic copepod (Lernaeopodidae) infesting the speckled trout (*Salvelinus fontinalis* Mitchill) at Gaspé, P.Q., and also taken from arctic charr (*Salvelinus alpinus*) collected at Sugluk Bay, northern Labrador. *S. salvelini* is found in the mouth of both hosts where it is firmly attached to the tissues of the roof and sides of the mouth and tongue. It does not occur on the gills, and in this respect is distinct from *Salmincola edwardsii* commonly found on speckled trout in North America. *S. salvelini* has a marked superficial similarity to *S. gibber*, from which it can be distinguished by the presence of a spine on the terminal segment and a papilla on the penultimate segment of the maxillipeds.

## Introduction

Records of parasitic copepoda infesting the speckled trout (*Salvelinus fontinalis* Mitchill) to the present time appear to be restricted to the two species, *Salmincola edwardsii* (Olsson) (Lernaeopodidae) and *Argulus canadensis* Wilson (Argulidae). The material described in the present paper constitutes a third and apparently undescribed species for which the name *Salmincola salvelini* is proposed.

*Argulus canadensis* Wilson has been reported from speckled trout at Cape Breton, where it infests many of the fresh-water fishes (Wilson, 6). *S. edwardsii* is a common and frequently serious parasite of the speckled trout and has a wide distribution on this continent (Davis, 1). In the Province of Quebec, *S. edwardsii* (recognised by fishermen as the bug, or "bebitte") is occasionally found on trout living under natural conditions; and in certain lakes (Spider Lake, Frontenac County, and adjacent small lakes to the south, as well as several lakes in the vicinity of Murray Bay, Charlevoix County) *S. edwardsii* produces or contributes to a significant mortality amongst the adult trout.

*Salmincola salvelini*, on the other hand, has been collected from speckled trout in the Provincial Fish Hatchery at Gaspé, and from arctic charr (*Salvelinus alpinus*) taken at Sugluk Bay, northern Labrador. The former specimens were sent to the author by Mr. R. C. Lindsay, Superintendent of the Provincial Fish Hatchery at Gaspé. The latter specimens were found in charr belonging to the collection of the Institute of Parasitology, Macdonald College. The specimens from both districts show similar variation in form and possess similar mouth parts.

In both cases the parasites were restricted to the mouth, being attached to the membrane lining the mouth and hanging with the body free in the buccal cavity, anterior to the insertion of the gills. Many specimens were

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attached to the tongue and alongside this structure; a smaller number occurred on the roof and sides of the buccal cavity.

*S. salvelini* shows a marked superficial dimorphism. From the one host it is common to obtain specimens with an orbicular, depressed trunk and others with a narrower, rectangular and deeper trunk. This dimorphism is apparently correlated with the position of attachment of the parasite in the mouth of the host, the flattened orbicular forms being attached to the tongue, while the rectangular forms are generally found attached to the sides or roof of the mouth. Several specimens, taken from the groove at the side of the tongue where they were subjected to mechanical pressure during closure of the mouth of the host, possess a normal cephalothorax and a markedly distorted trunk.

The orbicular forms have a superficial resemblance to *Salmincola gibber*, although the cephalothorax is not as wide in proportion to the width of the trunk as in this latter species. The mouth parts of the two species are also distinct. The first antenna of *S. salvelini* bears distally three spines which lack the segmentation and fringe of hairs shown for the corresponding structure of *S. gibber* (Wilson, 2). The second antennae of the two species are quite distinct from one another. In *S. gibber*, the dorsal ramus of this appendage is in the form of a large flattened claw bearing two spines on the concave margin. The same structure in *S. salvelini* is segmented, and consists of a broad basal segment bearing a squat papilla and a small spine, and a terminal segment in the form of a strong claw-like spine. The ventral ramus in *S. salvelini* is unsegmented and bears only a single simple spine, while the ventral ramus in *S. gibber* is segmented, having a large basal segment and a smaller terminal segment which is fringed with fine hairs. In addition, there is a small palp situated on the ventral aspect of the proximal portion of the appendage. This is not described for *S. gibber*.

The mandibles of both species are similar, although in *S. salvelini* the ventral edge of the shaft is straight, not concave as in the other species. The maxillae of *S. salvelini* lack the protuberance seen on this structure in *S. gibber* and are also distinct in having two of the distal spines mounted on inflated bases. The arms of *S. salvelini* are relatively narrower than in *S. gibber* and the dorsum of the cephalothorax can be clearly seen in lateral view.

The most readily observed distinction between the two species is found in the maxillipeds. The maxillipeds of *S. salvelini* bear on the terminal segment a small spine, and on the penultimate segment a broadly conical papilla. These structures are quite obvious without dissection and have no counterparts in *S. gibber*.

Wilson (5) has listed further a total of eight species of *Salmincola* from arctic waters, where it is apparent that the genus is well represented. The short and broad cephalothorax, the simple form of the trunk and the mushroom-like bulla of *S. salvelini* sets this species apart from other arctic forms with the exception of *S. gibber*, from which, as shown above, it is clearly

distinct on the basis of the mouthparts as well as minor relative proportions in form.

The present collection contains 22 specimens, five of which were taken from arctic charr collected at Sugluk Bay. The remainder were collected at Gaspé. Some of the material fixed in formalin is badly shrivelled and the measurements have been made from other material preserved in alcohol.

### Description

Genus *Salmincola* Wilson 1915.

Fixed parasitic copepods with the cephalothorax lacking a carapace and separated from the unsegmented, stout trunk by a groove or neck, with the maxillipeds inside of the second maxillae, the latter being longer than the cephalothorax and situated close behind the mouth tube.

*Salmincola salvelini* SP. NOV. Fig. 1, A-H.

The male has not yet been collected.

The females are of moderate size, the cephalothorax and trunk measuring 6.0 mm. in an average specimen. The cephalothorax (Fig. 1, B) is almost as wide as long and has slightly concave lateral margins. Between the proximal ends of the second maxillae the cephalothorax is swollen markedly, giving much the appearance of heavy shoulders when seen in lateral view. The cephalothorax is joined to the trunk by a short and broad "neck". The trunk is variable in shape; on the one hand many specimens have a trunk markedly flattened and circular in dorsal view, while many others have a more elongate, narrower trunk, rectangular when seen in dorsal view. In the latter specimens the trunk is not markedly depressed. The trunk lacks any posterior process. The egg-sacs are long and stout, generally approximating (in orbicular forms) the combined length of the cephalothorax and trunk, or exceeding it (in rectangular forms). The trunk and egg-sacs in specimens attached to the tongue are frequently deformed.

The first antennae (Fig. 1, D) are small, unsegmented, and tipped with three small spines. The first antennae are the only appendages, with the exception of the second maxillae, that are visible in a dorsal view of the cephalothorax. The second antennae (Fig. 1, E) are sturdy and laterally compressed. Segmentation is obscure. The ventral ramus of the second antenna is a simple conical extension ending bluntly with a smooth surface and bearing distally a single weak spine. The dorsal ramus is a two-jointed conical structure, the proximal segment being inflated and bearing ventrally a low conical papilla and a short spine. The distal segment forms a strong, conical, slightly asymmetrical cone. Close to the base of the dorsal ramus and on the lateral aspect, the proximal portion of the appendage bears a low, rounded protuberance covered with short spinules which form a hispid patch. Proximal to this hispid protuberance and also on the lateral aspect there is a small palp-like structure bearing five or more small spines. The terminal spines, and papilla of the dorsal and ventral rami are curved slightly so that their tips point away from the mouth tube.

The mandibles (Fig. 1, G) are short, broadest at a point half-way from their base, and beyond this taper sharply to the small rounded distal end. The ventral margin is straight. The teeth are few and recurved, increasing in size from the small distal tooth to the third, which is the largest. The two most proximal teeth are weak.

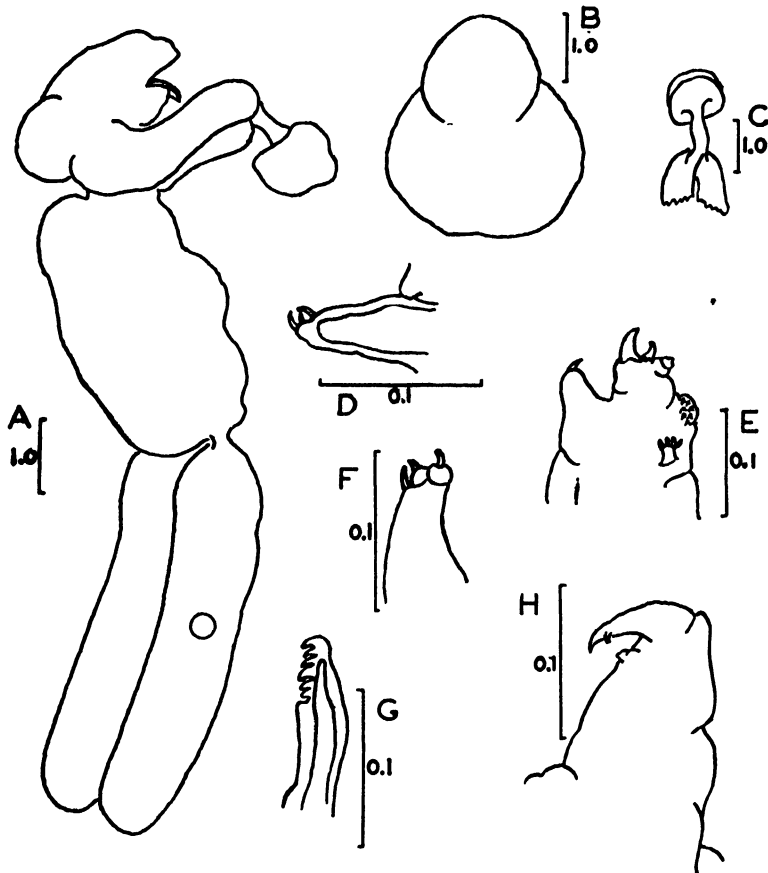


FIG. 1. A. Lateral view adult female *Salmincola salvelini*. B. Cephalothorax in dorsal view. C. Distal ends of second maxillae showing bulla. D. First antenna seen from the lateral aspect. E. Second antenna from lateral aspect, showing the proximally situated palp-like structure. F. The first maxilla from the lateral aspect. G. Mandible. H. The maxilliped from the ventral (posterior) aspect.

N.B. The scales are in millimetres.

The maxillae (Fig. 1, F) are small and short, bearing distally three small spines, two of which are mounted on bulbous expanded bases. The second maxillae vary from strongly curved, short "arms" approximately half the length of the trunk (orbicular forms), to long, slender, and almost straight "arms" subequal to the length of the trunk (rectangular forms). The bases of the second maxillae are not markedly swollen. The bulla is of moderate size, petioled and mushroom-shaped. (Fig. 1, C.)

The maxillipeds (Fig. 1, H) are stout. The penultimate segment bears on its lateral surface a broadly conical papilla tipped with a very fine spinule or hair. It is but little longer than the terminal segment, which has a concave margin and bears a strong spine on the medial surface.

The cephalothorax averages 2.5 mm. long by 2.35 mm. wide. The trunk is 4.5 mm. long by 3.0 mm. wide and 2.5 mm. deep in forms with a rectangular trunk, and 3.5 mm. long by 3.5 mm. wide and 1.5 mm. deep in forms with a circular trunk. The egg-sacs, in forms with a rectangular trunk, are 8.0 mm. long and 1.5 mm. wide, while in forms with a circular trunk the egg-sacs are slightly wider but only 5.0 mm. long. Eggs in the egg-sac are 0.3 mm. in diameter.

In all available material *S. salvelini* has been found attached to the tongue and tissues lining the buccal cavity back to the beginning of the pharynx only. The author is indebted to Mr. Lindsay for the statement that even in heavily infected trout, *S. salvelini* is not present on the gills. In this habit *S. salvelini* differs markedly from *S. edwardsii*, from which it may be distinguished on sight by the smaller size and different habit of the latter.

Wilson (4), in giving a list of new hosts for parasitic copepods, records the collection of *S. gibber* from the mouth and tongue of the charr (*Salvelinus alpinus alipes*) taken at Cairn Lake and Knochickalak Lake by the Mac-Millan Baffin-land expedition.

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# THE NORTHERN FOWL MITE (*LIPONYSSUS SYLVIARUM* C. & F., 1877)

## INVESTIGATIONS AT MACDONALD COLLEGE, QUE., WITH A SUMMARY OF PREVIOUS WORK<sup>1</sup>

BY DONALD CAMERON<sup>2</sup>

### Abstract

This paper gives a complete review of past work on the northern fowl mite. The generic name for the species is *Liponyssus* Kolenati, 1859. To show the geographical distribution of this mite, 20 bird and two mammalian hosts are given in systematic order. A seeming discrepancy in previous descriptions of the protonymph is figured and described. A description of the larva and of the males, resembling those of *L. bursa*, is given with figures. They were taken, with males of *L. sylviarum*, from fowls at Macdonald College, but all females taken from the birds are as described for *L. sylviarum*. Average duration of the egg stage is 30.4 hr. and of the larval stage 8 33 hr. at 100°-104° F. and 90-100% relative humidity. All attempts at artificial feeding failed. This mite does not aestivate and will not breed upon chicks. It multiplies rapidly, spreads readily from bird to bird, and survives long periods of starvation, but temperatures below 7° F. cause death in a short time, when away from the host. The high thermal death point lies between 104.2 and 108.5° F. The economic importance is uncertain but might be great. Control is cheaply and easily accomplished by the use of nicotine sulphate.

### Introduction

Since 1920, when Wood first collected the northern fowl mite (*Liponyssus sylviarum* Canestrini and Fanzago, 1877) from domestic poultry in the United States, much has been written about it but much work still remained to be done. In 1922, Caesar reported it from poultry at Guelph, Ontario, and since then it has appeared in other parts of Canada, where it has been reported as doing serious damage to the domestic hen.

The Quebec Department of Agriculture provided the funds for the following investigation, which were made at Macdonald College, Que., between October, 1935 and May, 1937.

In this paper the author has tried to review all past work and from this review and his own observations to clarify the synonymy and give as complete a host list as possible; distinguish between this mite and the tropical fowl mite (*Liponyssus bursa* Berlese, 1888); describe the morphology, life history and habits; determine the economic significance; and state the best means of control. A complete bibliography is also given.

### Synonymy

1877 *Dermanyssus sylviarum*; Canestrini and Fanzago. Atti ist. Veneto sci. 5 : 4 : 124.

1884 *Leiognathus sylviarum*; Canestrini. Atti ist. Veneto sci. 6 : 2 : 1573-1660.

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- 1885 *Leiognathus silviarum*; Canestrini, Prospetto dell' Acarofauna Italiana, 121.
- 1889 *Leiognathus sylviarum*; Berlese, Acari Myriopoda et Scorpiones hucusque in Italia reperta. 53 : 5 : 19.
- 1893 *Leiognathus sylviarum*; Berlese, ebenda, Ordo Mesostigmata. 22.
- 1920 *Liponyssus sylviarum*; Vitzthum, Arch. Naturgeschichte. 84 (A) : 6 : 27.
- 1922 *Liponyssus sylviarum*<sup>1</sup>; Hirst, Brit. Mus. Nat. Hist. Econ. Ser. 13 : 90.
- 1922 *Leiognathus sylviarum*; Ewing, Proc. U.S. Nat. Mus. 62 : 13 : 7.
- 1926 *Liponyssus sylviarum*; Vitzthum, Seckenb. Frank.-a-M. 8 : 30-39.
- 1931 *Liponissus sylviarum*; Vitzthum, Z. Parasitenk. 4 : 1 : 9-11.

The genus *Liponissus* was erected by Kolenati, in 1858, from a male specimen of *Dermanissus setosus* Kolenati, 1856. In 1859 he gave three drawings of the monotype, including a dorsal view of an adult and a ventral view of a nymph or (more probably) of a female. He also altered the spelling, at this time and thrice later, to *Liponyssus*, which spelling has since been retained (incorrectly according to Vitzthum (55) ) in the literature. In a letter to the writer, Dr. C. W. Stiles states that he had adopted *Liponyssus* on the basis that Kolenati's papers are inconsistent, that the earliest *nyssus* in this group is *Dermanyssus*, and that (since Kolenati in 1858 uses *nyssus* in six generic names and *nissus* in only one) the derivation of *nissus* is identical with that of *nyssus*. Thus *nissus* is clearly an error of transcription and therefore subject to correction.

It is no longer possible to identify the type species *Liponyssus setosus* Kolenati, 1859. Kolenati's only record is from a Siberian horse-shoe bat, *Rhinolophus clivosus*, and he handed over no co-types to the large museums. Besides, according to Vitzthum (55), his description does not agree with his figures and the latter are insufficient.

In 1877, a written description of a new species was published, and this species was named *Dermanyssus silviarum* by Canestrini and Fanzago.

Canestrini in 1884 formed a new genus *Leiognathus* for *Liponyssus arcuatus* Koch, *L. silviarum* C. & F., and *L. uncinatus* sp. n. He repeated the written description of *L. silviarum* in another publication (1885), and Berlese (3) redescribed this mite as *Leiognathus sylviarum* and added figures of dorsal and ventral sides of the female, and of the epistome, peritreme and chelicera. He repeated this description in 1893.

Vitzthum (53) redescribed the female with figures of ventral and dorsal aspects and the ventral plate, besides describing the protonymph for the first time, with figures of the dorsal and ventral aspects, peritreme and tarsus. He included *Leiognathus* Canestrini, 1884, in *Liponyssus* Kolenati, 1859, and so the species became *Liponyssus sylviarum* (Canestrini and Fanzago, 1877).

As sole difference between *Leiognathus* and *Liponyssus*, Ewing (19) states that in the females of *Leiognathus* "the body is constricted suddenly and is provided with an incomplete transverse groove behind the insertion of the

<sup>1</sup> The mite described by Megnin in 1891, under the name *Lophoptes palavinus*, as causing a special acariasis in Paduan fowls, is possibly *L. sylviarum* (Hirst, 26).

last pair of legs." According to Vitzthum (54), this cannot be verified in *Leiognathus sylviarum* unless the female is fully mature and gravid, and even then it is only suggested.

Vitzthum (54) states that the horn-shaped apophysis of the palpus-trochanter of the female of *Liponyssus sylviarum* (also a character of the genus *Ceratonyssus*), should it occur again in females of species having undivided dorsal shields, must cause further separation of the genera in question.

## Geographical Distribution with Hosts in Systematic Order

### A. TRUE HOSTS

#### Aves

Gallinae (fowl-like birds)

Phasianidae (poultry)

*Gallus domesticus* L. (domestic fowl)

U.S.A.—

Md., Beltsville  
Ill., Raymond

}—coll. Wood (59) det. Hirst (24) On fowls and inside  
(first as a variety of *L. bursa*, straws in nests.  
later as *L. sylviarum*).

Ind., Lafayette  
N.Y., (northern)  
Minn., Minnesota

—Troop (51) On fowls only.  
}—Bishopp (5) On fowls and in nests.

Ind., Bloomfield  
Ill., Harvel  
N.Y., Plattsburg  
Ohio, Oxford

}—Cleveland (15) (uncertain whether dealing with *L. sylviarum* On fowls and in nests.  
or *L. bursa*).

N.Y., Closter  
N.Y., Ithaca

}—Matheson (35) ?

Va.  
N.C.  
Fla.

}—Kaupp (30) On fowls and in nests.

Ohio, Wooster

—Cutwright (16, 17) (uncertain, On fowls and in nests.  
calls mite the "feather mite",  
sometimes "the tropical fowl  
mite" *L. sylviarum*)

Kansas, Manhattan

—Payne (42) (calls it *L. sylviarum* On fowls, in nests, on  
but Bushnell and Brandly dropping boards, in  
(9) call it *L. bursa*). cracks in roosts and  
walls.

Mass., Agr. College

—Payne (43) (calls it *L. sylviarum* On fowls, in nests, on  
of Cleveland (15) but later dropping boards, and  
was uncertain whether he had in cracks in roosts  
*L. bursa* or *L. sylviarum*) and walls.

Canada—

Ont., Guelph  
Ont., Port Dover

}—Caesar (10) On fowls and in nests.

B.C., New Westminster

—Spencer (48) On fowls and in nests.

Que., Macdonald College

—Maw (37) On fowls and in nests.

England—

Bedfordshire, Bletsoe  
Dorsetshire

—Hirst (27). ?  
—Taylor (50) On fowls only.

Columbae (pigeons and doves)

Columbidae (pigeons)

Europe  
England

}—Hirst (26) On birds only.

## Aves—Continued

## Pici (woodpeckers)

## Picidae (woodpeckers)

*Dryobates villosus villosus* L. (hairy woodpecker)

## Canada—

Que., Macdonald College —Rayner (44) On bird.

*Colaptes auratus auratus* L. (flicker)

## Canada—

Que., Macdonald College —Maw *et al* (38) On bird.

## Macrochires (swifts, goatsuckers, etc.)

## Micropodidae (swifts)

*Chaetura pelagica* L. (chimney swift)

## Canada—

Que., Macdonald College —Maw *et al* (38) On bird.

## Passares (perching birds)

## Tyrannidae (tyrant flycatchers)

*Tyrannus tyrannus* L. (kingbird)

## Canada—

Que., Macdonald College —Rayner (44) On bird.

## Sturnidae (starlings)

*Sturnus vulgaris* L. (European starling)

## U.S.A.—

— —Matheson (35) On bird.

Ohio, Wooster —Cutwright (as above for *G. domesticus*). On bird.

## Canada—

Que., Macdonald College —Rayner (44) On bird.

## Icteridae ("troupials")

*Molothrus ater ater* Bodd. (cowbird)

## Canada—

Que., Macdonald College —Maw *et al* (38) On bird.*Euphagus carolinus* Müller (rusty grackle or blackbird)

## Canada—

Que., Macdonald College —Rayner On bird.

*Quiscalus quiscula quiscula* L. (purple grackle)

## Canada—

Que., Macdonald College —Maw *et al* (38) On bird and in nest (heavy).

## Fringillidae (finches)

*Passer domesticus* L. (European sparrow)

## U.S.A.—

Md., Beltsville

Ill., Raymond

}—coll. Wood (59) det. Hirst (24) On birds (light), nestlings and nests (heavy).

— —Bishopp (5) On nestling.

## Canada—

Que., Macdonald College —Rayner (44) On bird and in nest.

## Hirundinidae (swallows)

*Progne subis subis* L. (purple martin)

## Canada—

Que., Macdonald College —Rayner (44) On bird.

*Hirundo erythrogastra* Bodd. (barn swallow)

## Canada—

Que., Macdonald College —Maw *et al* (38) On bird.

## Mniotiltidae (wood warblers)

*Dendroica aestiva aestiva* (Gmelin) Baird (yellow warbler)

## Canada—

Que., Macdonald College —Maw *et al* (38) On bird.

## Motacillidae (wagtails and pipits)

*Motacilla alba* L. (European white wagtail)

## Russia—

—Hirst (24) In nest.

## Mimidae (mocking birds)

*Dumetella carolinensis* L. (catbird)

**Aves—Concluded****Passares (perching birds)—Concluded**

- Canada—  
 Que., Macdonald College —Rayner (44) On bird and in nest.  
*Sylviidae* (kinglets and gnatcatchers)  
*Sylvia atricapilla* L. (blackcap warbler)  
 Italy—  
 Pisa —Canestrini and Fanzago (12) On bird and in nest.  
*Sylvia curruca* L. (lesser whitethroat)  
 Germany—  
 Weimar —Vitzthum (53) In an old nest.  
*Turdidae* (thrushes and their allies)  
*Planesticus migratorius migratorius* L. (robin)  
 Canada—  
 Que., Macdonald College —Rayner (44) On bird and in nest.  
*Turdus merula* L. (blackbird)  
 Italy—  
 Portici —Leonardi (33)

**B. ACCIDENTAL HOSTS (BITING BUT NO APPARENT MULTIPLICATION ON HOST)****Mammalia****Rodentia****Muridae**

*Dicrostomys hudsonicus* (Labrador collared lemming) det. Anderson.

**Canada—**

- Que., Macdonald College —coll. W. E. Whitehead, Nov. 19,  
 1935. Det. H. E. Ewing,  
 1933 (unpublished).

**Primates****Hominiidae**

*Homo sapiens* (man)

**U.S.A.—**

- —Riley and Johannsen (45, p. Bites caused pruritis  
 265). (intense itch).  
 Ill., Raymond —Wood (59) Bites.

Wood (59), at Raymond, Ill., reports negative records for the brown thrush, song sparrow, blackbird, redheaded woodpecker, screech-owl, quail, robin, mouse nest, and mole.

Bishopp (5) states that *L. sylviarum* has never been recorded in southern latitudes. However, the first record came from Pisa, Italy, in 1877, and Kaupp (30) cites records from Maryland, Illinois, Indiana, Virginia, California and Florida.

Payne (42) gives negative records for the English sparrow at Manhattan, Kansas, and at the Massachusetts Agricultural College (43). He states that climate seems to be no barrier to the distribution of *L. sylviarum*. However, it is uncertain whether he was working with *L. bursa*, *L. sylviarum*, or both; and Bushnell and Brandly (9), also at Manhattan, Kansas, declare that the mite reported by Payne as *L. sylvarium* was probably *L. bursa*.

The tropical fowl mite *Liponyssus bursa* was first reported in 1888 from poultry in Buenos Aires, Argentina, South America. Since then Hirst (22) reports it on poultry in South America, Africa, Mauritius, China and India. Records are also given of specimens from starlings, sparrows, the "hibon" (Comoro Islands), from a lizard and from man (India and Zanzibar).

Hirst (23) and Roberts (46) report it from poultry in Australia, and the latter also reports it from pigeons and sparrows. Definite instances of attacks upon man are reported from China by Hirst (25), from Australia by Cilento (13) and Roberts (46). The latter states that *L. bursa* attacks any animal in the house, but only lives ten days away from the host and cannot breed in the meantime.

During the winter months, wild birds found around the poultry plant at Macdonald College are limited to starlings and sparrows. *L. sylviarum* has been recorded from both these hosts (Rayner (44)), but the records were made during the summer months. The writer examined sparrows and starlings during the winter months: 16 sparrows yielded nothing, one of 13 starlings carried a very light infestation of *L. sylviarum*, and 30 pigeons gave negative records, as did 7 rats.

### Differences between *L. sylviarum* and *L. bursa*

*L. bursa* has three pairs of hairs on the sternal plate; two pairs of long hairs at the posterior tip of the dorsal shield; and (in the male) no transverse line posteriorly, on the ventral plate, in front of the anal plate. *L. sylviarum*, on the other hand, has but two pairs of hairs on the sternal plate; one pair at the posterior tip of the dorsal shield; and (in the male) has a transverse line present, posteriorly, on the ventral plate, in front of the anal plate.

Hirst (26), although he points out the above differences, states that perhaps *L. bursa* is a variety of *L. sylviarum*. The significance of this should not be overlooked, since *L. bursa* is suspected of disease transmission from rodents to man, and *L. sylviarum* is here reported from a Labrador collared lemming.

Among mites collected from fowls at Macdonald College, the writer found all the females to agree with the above-mentioned figures and descriptions of *L. sylviarum*; but while some of the males were as figured for *L. sylviarum* by Hirst (26), others were more like those which he figured as being *L. bursa* (cf., Fig. 1B in this paper, with Fig. 69 by Hirst (26)).

## V. Morphology, Life History, and Habits

### A. MORPHOLOGY

#### General

A translation from the Italian of the original written description of *L. sylviarum*, Canestrini and Fanzago (12), is as follows:

"Body oval, elongate; slightly restricted behind the shoulders, and rounded and slightly notched at the posterior margin. A small seta on the shoulder, on each side, and another small one in front of this. The whole margin of the abdomen bears moderately stout setae, which increase in robustness from front to rear, the last pair being situated on the posterior margin, above the anus and extending posteriorly. These two setae are distinguishable from the others by their pronounced extra length. The abdominal surface is smooth. The legs are all covered with uniform setae except in the fourth pair, which, in the penultimate segment, are consistently longer and more curved."

"The colour, of our example, bleached to a uniform white in alcohol.

"Length: one mm.

"Habitat: found on *Sylvia atricapilla* (collector: Professor Richardi)."

In 1884, Canestrini gave a written description of the male and female, with five drawings to illustrate the latter sex.

Berlese (3, 4) gives a written description of the female with figures. He states that Canestrini (11) probably studied a replete nymph and not a female.

Vitzthum (53) gives descriptions and figures of ventral and dorsal views of female and protonymph, and criticizes the description of the female by Berlese. He states that Berlese failed to notice certain hairs on the dorsal shield and on the soft-skinned dorsal area, that the peritreme was wrongly described, and that, on the ventral side, only the anterior half of the genital plate was noted. Vitzthum also says that it is doubtful if Canestrini had seen the male, and that the protonymph can no longer be known as the female.

The egg, larval, protonymphal, deutonymphal and adult stages are mentioned by Wood (59). His material was identified by Hirst (25), who showed the morphological differences between *L. bursa*, *L. sylviarum* and *L. bacoti* (26).

In 1923, Cleveland figured and described the egg, larval, protonymphal, deutonymphal and adult stages of a mite which he states is either *L. bursa* or *L. sylviarum*, but his figures are scarcely adequate.

Vitzthum (55) states that "Von keiner *Liponissus*—Art kennt man eine Deutonympha. Dieses Stadium scheint übersprungen zu werden", and "Eine Protonympha dagegen besitzen alle Liponissiden und wahrscheinlich ist dies das Stadium, in dem sie zur Welt kommen. Eier legen sie keinesfalls, und eine frei lebende Larva ist noch nicht gefunden worden."

Maw, Whitehead and Bemont (38) give a photo of egg masses on feathers, and state "in colour the adults are darkish brown which shows some variations, with a distinct colour pattern which also varies apparently with age".

### *Egg*

Vitzthum's statements of 1931 contradict those made by previous workers. For photographs of egg masses see Wood (59) and Maw, Whitehead and Bemont (38). Cleveland (15) gives a figure of the egg of either *L. bursa* or *L. sylviarum*.

The writer has observed oviposition by *L. sylviarum* females kept in isolation in small vials, and larvae have been seen in the act of hatching from the eggs.

### *Larva*

An accurate figure is given here (Fig. 1A) of the dorsal aspect of a larva of *L. sylviarum* prior to moulting to the nymphal stage. This larva is colorless, six-legged, and has no plates on either ventral or dorsal surfaces. At the posterior end of the body are three pairs of stout bristles of equal length, and smaller bristles cover the rest of the body and legs. The chelicerae are non-functional, and the first pair of legs seems to be considerably longer than those of the nymphal stage. The developing chelicerae, palps and four pairs of legs of the nymphal stage are shown in this figure, and the fourth pair has just begun to straighten out and break through the larval skin.

*Protonymph*

The pair of long bristles, which Vitzthum (53) shows as arising from the posterior end of the protonymph, are found by the writer to arise from the posterior dorsal shield. The latter (Fig. 1C) is also different in shape to that figured by Vitzthum.

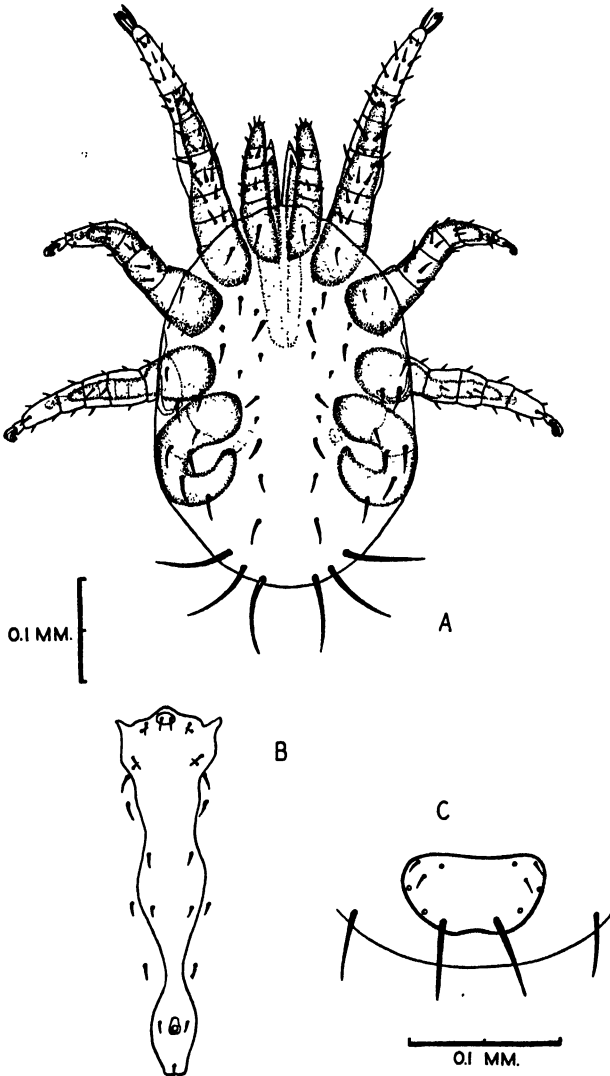


FIG. 1.

*Deutonymph*

Apparently no one has accurately figured or described this stage, and since the writer can find no form which he can positively identify as the deutonymph, he does not feel prepared to say at present whether there is such a form.

### Adult

See Vitzthum (53) for accurate figures and a description of the female.

Hirst (26) has figured and described the differences between both sexes of *L. sylviarum* and *L. bursa*. As already mentioned, the author has taken, from fowls at Macdonald College, some males resembling those figured by Hirst as *L. sylviarum* and others which are more like his figures of *L. bursa*.

Fig. 1B shows the ventral plate of a male mite, and is more like Hirst's figure for *L. bursa* than that for *L. sylviarum*. In shape it is less angular than *L. bursa*, but the margin is more sinuous; just in front of the anus the plate is narrower than that of *L. sylviarum*, and there is no indistinct suture present.

In his figure of the male of *L. sylviarum*, Hirst (26), although he does not remark upon it in his description, shows eight pairs of hairs on the ventral plate anterior to the anus, while in *L. bursa* the eighth pair are shown at the sides of, and not on, the ventral plate. In Fig. 1B of this paper it will be seen that the second, third, sixth and eighth pairs of hairs arise off the ventral plate.

All females examined were those of *L. sylviarum*.

### Egg

#### B. LIFE HISTORY

Oviposition occurs on the fowl or on the inside of straws in the nest, and the eggs adhere by means of a sticky substance, according to Wood (59). Troop (51) and Taylor (50) found them on the fowl only, and Caesar (10) states that they are found at the base of the feathers. Hatching occurs in three days off the host, according to Wood (59). Cleveland\* (15) found that 36 to 96 hr. were required, while Cutwright\* (17) agrees with Wood.

In the case of *L. bursa*, the eggs are laid away from the host, according to Hirst (22).

In January, 1936, the writer made preliminary experiments which indicated that the duration of the egg stage was from 30 to 31 hr. Accurate experiments were then made as described below.

Oviposition occurred in a dark incubator at a temperature of 100–104° F. Several females were placed in a tube plugged at the mouth by a wad of wet cotton wool wrapped in wet black silk. A similar wad was placed at the foot of the tube so that the relative humidity, inside the tube, was 90 to 100%. Examinations were made very 20 min. by means of electric light and a hand lens. Each egg was transferred by a camel's hair brush to a separate tube similar to the one in which it was deposited, where it was observed during hatching. The larval stage was observed until the time of moulting.

No female was observed to lay more than one egg in captivity; and since females could not be fed and recovered, the number of eggs laid per female was not determined. The mites congregated at certain points in the tube and laid their eggs in clumps. Deposition of an egg was almost instantaneous, but each female remained over her egg, or, if disturbed, made a short "tour

\* In this and subsequent references marked with an asterisk, there is doubt whether *L. bursa* or *L. sylviarum* was described by the author.

of inspection" and returned to the egg which she "fingered" alternately with left and right fore-legs.

When hatching, the egg splits in the horizontal plane across the posterior end and along both sides for over half its length. This splitting is brought about by the larva, which pushes backwards against the posterior end of the egg and then backs out of the shell. The average duration of the incubation period was 30.4 hr. (see Table I).

TABLE I  
DURATION OF EGG AND LARVAL STADIA

Time of oviposition	Duration of egg stage, hr.	Duration of larval stage, hr.	Total time, hr.	Remarks
13/1/36				
1. 5:30 p.m.	27.75	8.75	36.75	
2. 6:00 p.m.	27.25	Died	—	
14/1/36				
3. 12:30 p.m.	29.5	9	38.5	Egg deposited in drop of water.
4. 12:30 p.m.	32.25	7.75	40	Struggle to hatch.
5. 1:30 p.m.	30.25	8.25	38.5	Egg deposited in drop of water.
6. 1:30 p.m.	34	7.75	41.75	Hatching took 15 min.
7. 1:30 p.m.	33.75	8	41.75	Egg deposited in drop of water.
8. 2:30 p.m.	28.75	Died	—	Egg deposited in drop of water.
9. 6:30 p.m.	30	7.25	37.25	
10. 6:30 p.m.	30.5	10	40.5	
Average	(10) 30.4	(8) 8.33	(8) 39.34	

### *Larva*

The larva does not feed, and moults in 17 hr., according to Wood (59). Cleveland\* (15) states that the larva moults in less than a day. Preliminary experiments, made by the writer, gave the larval stage as 9 hr.; more accurate experiments, described above, indicated that the average duration of the larval stage is 8.33 hr. (Table I).

The larva may remain quiescent beside the egg or (presumably to find more favorable conditions) may move to a different location. Its movements are sluggish, and activity seldom occurs for long. At the posterior end of the larva, two bud-like structures appear and grow into the fourth pair of legs, which break through the larval skin and enable the protonymph to emerge backwards through this slit.

### *Protonymph*

Since the protonymphs could not be induced to feed artificially, and since they could not be recovered as identified specimens when placed on a clean bird, the writer has failed to work out the life history from this point onward. According to Wood (59), the moult occurs in one to two days, or (Cleveland (15)) in two to three days.

Attempts to feed the protonymphs artificially were made as follows:

1. Defibrinated hen's blood was placed in a tube over the mouth of which a membrane was stretched. The tube was inserted through a cork which was fitted into one end of a glass cylinder containing starved mites. The other end of the cylinder was covered with black cloth. The apparatus was placed in the dark in an incubator at the temperature of the bird's body (100–104° F.). The membranes used were fresh sparrow skin, fresh starling skin, fresh chicken skin, fine parchment, fine rubber membrane, and collodion membrane. The mites would not feed.

2. A small area near the tail head of a live bird was cleared of feathers and ringed round with vaseline within which starved mites were placed. They refused to feed, some even crossing the vaseline to reach the shelter of the feathers.

3. A small area beneath the wing of a live bird was cleared of feathers, and a rubber suction cap containing starved mites was attached. Even after several hours, the mites did not feed.

### *Deutonymph*

Moulting occurs in three to four days (Cleveland\* (15) ). Vitzthum (55) states that "Von Keiner *Liponissus*—Art Kennt man eine Deutonympha."

For reasons already stated, the writer is not prepared to say whether there is a deutonymphal stage.

### *Adult*

There is no information on the duration of this stage.

## C. HABITS

### *General*

Usually, moulting occurs on the host (Wood (59), Troop (51), Taylor (50) ). Feeding occurs both day and night (Wood (59), Cleveland\* (15), Maw (38) ), the mites being found on the body of the fowl at all times (Troop (51), Bishopp (5), Cutwright\* (16), Taylor (50) ). Mites are found in the nest as well (Caesar (10), Bishopp (5), Cutwright\*(16) ), and as many as 50 mites on a new-laid egg is a characteristic sign of infestation (Cutwright\* (16) ). Payne\* (42) and Maw (38) state that mites are found in the nests and on the dropping boards, roosts, and in the cracks in the walls of poultry houses. According to Cleveland\* (15), they do not breed in such places, and Wood (59) states that although live mites may be found on loose feathers in the shade, none are found alive on grass or in sunny places. Some of the mites, on a loose feather placed on the back of a bird standing in the sun, died before they could crawl beneath the hen's plumage.

Caesar (10) noted that mites come to the surface of the bird's plumage and bask in the sun, and Maw (36, 37) states that they are often seen on the surface of a hen's plumage, especially if the bird is brought into a warm atmosphere.

On the fowl, mites seem to prefer the vent region (Wood (59), Caesar (10), Hirst (27), Maw (38) ), and accumulate on a few feathers rather than on

many (Wood (59), Hirst (27) ), although in heavy infestation they spread to other parts of the body (Wood (59) ).

Mites feed intermittently, in patches the size of "a quarter", according to Wood (59).

Seldom are mites found on young chicks—probably due to lack of downy feathers—and an attempt to infest such chicks failed (Wood (59) ). An attempt to infest 12-week-old White Leghorn cockerels failed, although mites were placed in the birds' feathers and on the roosting boards and floor of the house (Maw (38) ). Payne\* (42), on the contrary, found mites on all ages and both sexes of chickens and states that they thrive on capons.

This parasite multiplies very rapidly (Hirst (27) ), the entire life cycle being 8 to 12 days (Cleveland\* (15) ).

After exposure in a bottle to a temperature of 7° F., or after 18 days in a bottle at laboratory temperature, mites survived (Caesar (10) ). Cleveland\* (15) and Maw (38) remark that mites live a long time away from the host, and Payne\* (43) noted that the survival period was from two to three weeks at laboratory temperature. Twinn (52) reported that infestation by *L. sylviarum* of a church at Bell's Corners, Ontario, occurred after nestlings had flown from the numerous nests around the eaves. This infestation disappeared two or three weeks later.

In the late fall, after poultry is in winter quarters, mites appear. Occasionally they leave a bird almost as suddenly as they attack it (Payne\* (42) ), and some hens may be heavily infested while others seem to be free of parasites (Wood (59), Payne\* (43) ). Apparently fowls that dust themselves most are freest (Wood (59) ). Some mites readily leave an infested fowl if the latter is handled (Wood (59), Taylor (50), Maw (37) ) and may bite through tender human skin (Wood (59) ). Twinn (52) states that numbers of *L. sylviarum* crawling over his hands did not bite.

As compared with the above-mentioned habits of *L. sylviarum*, Hirst (22) states that *L. bursa* infests the nest and surroundings of fowls and only attacks the fowls when food is required. When the birds leave the nest, the mites attack any animal or person in the house, but live only 10 days off the host and cannot breed in the meantime.

Maw *et al.* (38) believe that *L. sylviarum* aestivates during the summer months. This belief is apparently based on the fact that mites were not noticed upon birds during the summer months (although no specific search seems to have been made on the birds). During the summer, however, *L. sylviarum* was found on wild birds and in their nests.

From verbal reports it appears that *L. sylviarum* first appeared at Macdonald College in the fall of 1930, on seven White Leghorn male birds which had just arrived from the Central Experimental Farm, Ottawa. These birds were washed in a sulphur bath, but the infestation persisted; after spending the winter (1930–31) in a colony house, the birds were distributed over the college poultry plant and were used for breeding purposes. Since this time, sporadic outbreaks of mites have occurred.

### *Aestivation*

Investigations were made to discover if *L. sylviarum* aestivates. During the fall of 1935, poultry houses which had been infested the previous spring were thoroughly examined. Material was taken from under and on floors, from between the walls, from cracks in roosts and from the roof, subjected to heat in a Berlese funnel, and chilled in an incubator. No signs of *L. sylviarum* were observed.

After birds had been placed in their winter quarters, an infestation of *L. sylviarum* was found on pullets. No mites were found anywhere except on the birds. Of 51 birds, 15 were infested (one extremely heavily and the others to varying degrees). By January 11, 1936, all but one were infested to a greater or lesser degree.

During the summer months of 1935 these pullets had been in contact with other birds in the college orchards and possibly with wild birds. Probably one or more of the pullets picked up mites from these sources, and the mites spread to the other birds during the winter.

The following experiments were made to determine if aestivation occurs:

Experiment 1. Nine male birds were used. All birds happened to be infested with body lice (*Eomenacanthus stramineus*), which were killed by treating with sodium fluoride, the birds then being cleansed of the insecticide.

The birds were kept in individual coops under similar conditions in an empty pen. Three were placed on one side of the pen and used as controls, six were placed on the opposite side and infested with *L. sylviarum* (on March 7, 1936) by placing infested feathers in their coops and brushing the birds with them. This was repeated until all six birds were infested to a greater or lesser degree.

One bird died on April 2. The remaining five were still infested on June 15. At this time mites were found crawling all over the pen, and some attacked and infested two control birds, the third having died the previous day. On July 18, another of the controls died. The remaining six birds were now all heavily infested. One died on July 22, and one of the remainder was placed outdoors until October 1, (the cage being covered during cold or wet weather). On August 15, another bird died. Its tracheae were blocked with mucus, and the mite infestation was very heavy. Another bird died on October 21. It and the three live birds were still infested, but the bird which had remained outside from July 22 to October 1 was now carrying very few mites and showed no signs of infestation on November 9.

The other two birds remained infested until treated with nicotine sulphate on December 10 and 21 respectively, when the experiment was terminated.

Experiment 2. On March 11, 1936, a heavily-infested bird was placed in a vacant fumigation chamber at a temperature of 93° F. A dish of water was supplied. Twenty-four hours later many mites were discovered on the water, while many more were found in folds in the paper beneath the bird. The paper was removed to an incubator at 78° F. and relative humidity of 50 to 70%. It was stored for about seven months and examined. The mites were shrivelled and would not revive at lower temperatures.

Experiment 3. All stages of mites were left in an incubator at 60–65° F., with damp debris and a dish of water, from May 4 to October 6, 1936. The mites were then apparently dead, and did not revive after cooling to 30° F.

Experiment 4. On April 10, 1936, a White Leghorn pullet was placed in a wire cage, underneath which was a double bottom of beaverboard. Holes were punched in the upper layer, and some cotton wool was placed between the two bottoms. The whole was set on four small blocks in a shallow pan, which was kept full of water throughout the experiment. The environmental temperature was about 40° F. Mites could not escape from this cage, since tests have shown that they will not cross water. The pullet was heavily infested with mites the next day, and was kept in this cage until it died during the first week in June, with many live mites still actively feeding and breeding upon it. The cage was kept with all debris in it and surrounded by water until October 6. The false bottom was then examined thoroughly, but all mites were dead and none recovered at temperatures of about 105° F.

From the above results one may conclude that *L. sylviarum* does not aestivate, and it is possible that birds, when outside during the summer, get rid of most of their mites due to increased health and greater cleanliness; e.g., when taking dust baths a bird may shake off many of its mites, and leave them to perish in the hot sun.

Examinations of birds under natural conditions at Macdonald College revealed that some carried heavy infestations of *L. sylviarum* during June, 1937. In the first week of October, 1937, 10% of a flock of 10,000 White Leghorns at Hartford Mills near Cornell University, Ithaca, N.Y., were found to be heavily infested with this mite by Dr. Robert Matheson, who very kindly took the writer with him on a visit to this flock. The seasons at which these natural infestations occur are further indications that the mite does not aestivate but remains upon certain birds throughout the summer.

#### *Infestation of Baby Chicks*

On March 19, four chicks (19 days old) were placed in a vacant fumigation chamber at 100° F. with a gentle current of air flowing through it. A dish of water and some "Lakko" chick feed were supplied. Several attempts were made to infest these chicks by brushing them with infested feathers, by covering the floor with infested feathers, and by placing the chicks for short periods in a paper bag containing infested feathers, at 71.6° F., but mites could not be induced to breed upon the chicks, although some fed upon them. The chicks were left in these infested surroundings. Two weak chicks died on the fourth day, but the other two lived and thrived until removed six weeks later, when they showed no traces of mites.

#### *Progress of Infestation*

On Dec. 21, 1936, one of 19 Barred Plymouth Rock pullets, No. 932, was found to be carrying a very heavy infestation of mites, while the others were apparently free. In three pens of Barred Plymouth Rock females, all but six birds carried a very heavy infestation of lice, as did many of the White Leghorn females in two pens. The rapidity of spread and fluctuation of

TABLE II  
RAPIDITY OF SPREAD AND FLUCTUATION OF INFESTATION

No. of bird	1936		1937					
	Dec. 21	Dec. 24	Jan. 3	Jan. 7	Jan. 16	Jan. 22	Jan. 29	Feb. 4
932	v.v. heavy	v.v. heavy	v.v. heavy	v.v. heavy	v.v. heavy	light	light	light
945	-	light	f. heavy	f. heavy	f. heavy	v. heavy	v. heavy	heavy
946	-	light	v. heavy	v. heavy	v. heavy	v.v. heavy	v.v. heavy	light
929	-	light	light	f. heavy	f. heavy	f. heavy	f. heavy	f. heavy
943	-	light	light	f. heavy	f. heavy	heavy	v.v. heavy	f. heavy
942	-	light	light	light	light	light	f. heavy	f. heavy
935	-	light	light	light	light	v. light	v. light	v. light
936	-	light	light	light	light	light	f. heavy	f. heavy
931	-	light	light	light	light	light	v.v. light	v. light
941	-	light	light	light	light	light	f. heavy	f. heavy
948	-	-	v. light	v. light	v. light	v. light	f. heavy	f. heavy
942	-	-	v.v. light	v.v. light	v.v. light	v.v. light	f. heavy	f. heavy
950	-	-	v. light	v. light	v. light	v. light	light	light
937	-	-	-	v. light	v. light	v. light	v. light	light
938	-	-	-	-	light	v. light	v. light	v.v. light
940	-	-	-	-	-	v.v. light	v.v. light	v. light
933	-	-	-	-	-	-	f. heavy	f. heavy
934	-	-	-	-	-	-	light	light
949	-	-	-	-	-	-	v.v. light	clean
944	-	-	-	-	-	-	v.v. light	clean

v = very; f = fairly.

infestation in this house was recorded. The results are given in Table II. These show that infestation spreads rapidly from bird to bird, and that the degree of infestation increases rapidly on some birds, remains fairly constant on others, while certain birds keep clean.

#### *Length of Survival Period Away from Host*

Experiments were made to determine how long *L. sylviarum* will survive away from its host. Mites were subjected to the low temperatures of an empty laying house, the fairly constant temperature of the laboratory, and a series of constant high temperatures.

#### A. Low Temperatures

(i) Preliminary experiments. Mites in a glass tube were exposed for one hour at 8° F.; all died even when subsequently held at 70° F. On the other hand, exposure at 14° F. for one hour seemed to do no harm, since all mites recovered normal activity after 30 to 60 min. at 70° F.

(ii) On Dec. 29, 1936, mites were placed in small glass cylinders, both ends of which were closed by black cloth. The cylinders were divided into three groups; Group I was placed amongst the roof straw of a poultry house, Group II in holes punched in the Insulux material of the walls, and Group III in the floor debris, which had remained undisturbed since the fowls left it in spring. Each group consisted of the following: mites at all stages, egg masses, larvae, protonymphs, unfed adults, and replete adults. There were two cylinders of each of these, and feathers were added to one of each pair.

A hygro-thermograph was placed on the floor beside Group III and was covered lightly with floor debris. A maximum-minimum thermometer was suspended on the wall between Groups I (roof) and II (wall).

Mites on infested birds in the same house served as controls.

After 24 hr. at temperatures ranging between 16 and 28° F. and relative humidities of 64 to 84%, most of the mites in all stages recovered following 2 to 3 hr. in the laboratory at 70° F. and relative humidity of 20 to 50%. Eggs, however, were not observed to hatch. A few controls had also died.

Eggs and dead mites of all stages were replaced by fresh specimens in all groups and the cylinders were replaced. During the next 153.5 hours, the temperature varied from 10 to 46° F. with relative humidity of 54 to 100%. The mites were then brought into the laboratory at 70° F. and relative humidity of 20 to 50%, and left there under observation for two days. No mites of any stage recovered in Groups I and II, but in Group III, which had been in more humid surroundings, a few adults recovered normal activity. The tubes were placed for 18 hr. at 105.8° F. and relative humidity of 90 to 100%. There were still no signs of recovery after 10 days in Groups I and II, but a few more adults recovered in Group III. No eggs or immature stages had recovered. Controls were still alive at this time.

(iii) Cylinders of mites were divided into three groups. In each group, ten cylinders each contained about 100 active mites and many eggs, while an equal number contained, in addition, roof debris (Group I), wall debris (Group II), or floor debris (Group III).

Groups I, II and III were placed in the roof straw, in holes in the walls, and in the floor debris, respectively. Mites on infested birds were used as controls.

A hygro-thermograph and maximum-minimum thermometer were used as in (i).

The experiment lasted for 10 days (Jan 30–Feb. 9, 1937). The temperature fluctuated from 8 to 36° F. and the relative humidity from 50 to 90%. The cylinders were then placed at 96–104° F. and 90–100% relative humidity for about 24 hr., during which time they were kept under observation. About two-thirds of the adults in Group III recovered, most of which were in the cylinders without debris. No other mites survived and no eggs hatched. Mites were still present on the birds.

(iv) Ten cylinders of mites on feathers were placed in the floor debris of the above poultry house on Feb. 22, 1937.

Temperature and humidity records were kept as above.

Three weeks later, all mites were dead. The floor temperature, during this period, had fluctuated between 7° and 48° F. and the relative humidity between 40 and 77%.

Controls were still alive and normal.

## B. Laboratory Temperature

All stages of mites were kept with feathers in bottles at 70° F. (bottles opened momentarily from time to time, to keep air fresh). Of the mites in

five bottles kept 19 to 30 days, none survived. In one bottle kept 11 days, 25% of the fed adults survived.

All stages of mites, kept on the surface of water in a dish, were dead in 11 days at 70° F.

Controls on the fowls under the same conditions were still alive.

#### C. High Temperatures.

All stages of mites, in each of 3 bottles, were dead in  $\frac{1}{2}$  hr. at 122–126° F.

All stages died in 1 hr. in a bottle at 116–122° F.

All stages died in  $3\frac{1}{2}$  hr. in a bottle at 111.2° F.

All stages died in 5 hr. in a bottle at 110.3° F.

All stages died in 3 hr. in a bottle at 108.5–110.3° F.

All stages died in 5 hr. in a bottle at 108.5° F.

All stages survived at 104.2° F., and therefore the high thermal death point of this species lies between 104.2° F. and 108.5° F.

## VI. Economic Significance

### Historical

#### A. POULTRY

Infestation of poultry by a mite definitely identified as *L. sylviarum* is confined to the domestic hen (*Gallus domesticus* L.). All workers state that it causes economic loss to a greater or lesser degree (*cf.* previous literature). Heavy infestation may cause the fowl to die (Wood (59) ), or to become so weak that it is unable to stand (Taylor (50) ). Maw (37) asserts that vitality is reduced through loss of blood. Besides, egg production is reduced (Hirst (26, 27) ), and these mites cause bloody scabs on the skin of the bird (Hirst (27) ) owing to secondary infection of the bites (Whitehead (56) ). Scabs appear chiefly on the back, wing joints and tail head (Maw (38), whose paper contains photographs showing scabs on plucked fowls; Payne\* (42) ). Cleveland\* (15) observed bloody scabs on the skin of the bird.

Some birds are more heavily infested than others, according to Wood (59). Wood thinks that fowls which dust themselves most are freest, and he and Maw (38) state that cocks seem to carry a relatively heavier infestation than hens. Payne\* (43) states that while some fowls may be heavily infested others seem to be free of parasites.

Wood (59) found few mites of *L. sylviarum* on young chicks, and Bishopp (5) states that none are found on baby chicks. On the other hand, Cleveland\* (15) says that young birds are infested, and Payne\* (43) asserts that the death loss in young chicks is very heavy, and all ages of males, females and capons are attacked.

Transmission of spirochaetosis from bird to bird by *L. bursa* was suggested by Hirst (22) and Roberts (46). In 1936, Brody proved that "fowl-pox" may be transmitted from bird to bird if *L. sylviarum* mites, fed on an infected bird, are crushed and inoculated into a healthy bird 4 days after the infective meal; but adult mites, fed on a healthy bird 4 and 11 days after their last association with a diseased bird, did not cause pox.

*Post Mortems*

Post mortems were performed on the following mite-infested birds during 1936.

(1) Feb. 19, White Plymouth Rock pullet.

Liver—slight lesions of *Histomonas meleagridis*.

Caecum—light infestation of *Heterakis gallinae*.

Duodenum—inflamed 3 in. from gizzard, owing to infestation with *Capillaria* spp. (diarrhoea symptoms evident before death).

Oviducts—highly fibrous-diseased.

Other organs—normal.

*L. sylviarum* infestation heavy (duration unknown). Death was not due to mites, but might have been caused by the *Capillaria* spp. or the diseased condition of the oviducts, or both.

(2) Feb. 22, White Plymouth Rock pullet.

Duodenum—very haemorrhagic (diarrhoea symptoms evident before death)—probably *Capillaria* spp., as many birds in the same pen showed diarrhoea symptoms and eggs of *Capillaria* spp. were found in the faeces of some.

Infestation with *L. sylviarum* very light; death probably not due to mites.

(3) Feb. 22, White Plymouth Rock pullet.

Bird too fat.

Heart slightly enlarged.

Trachea very haemorrhagic and blocked with bloody exudate.

Caecum—a few *Heterakis gallinae* present.

*L. sylviarum* in small numbers. The bird died of bronchitis.

(4) Feb. 27, White Plymouth Rock pullet.

Bird too fat.

Heart enlarged.

Tracheae very haemorrhagic and blocked with bloody exudate.

Liver—a few lesions of *Histomonas meleagridis*.

Caecum—a few *Heterakis gallinae*.

Duodenum slightly inflamed.

A few *L. sylviarum* present. The bird died of bronchitis.

(5) Feb. 25, White Plymouth Rock pullet.

Findings as for (3), but *L. sylviarum* infestation heavy (duration unknown). Death probably due to bronchitis.

(6) Feb. 27, White Plymouth Rock pullet.

Findings similar to (4). *L. sylviarum* infestation heavy (duration unknown). Death due to bronchitis.

(7) Feb. 27, White Plymouth Rock pullet.

Findings similar to (4) with a similarly light infestation of *L. sylviarum*. Death due to bronchitis.

(8) March 8, White Plymouth Rock pullet.

Bird emaciated.

Duodenum very haemorrhagic.

Very heavy infestation of *L. sylviarum* (duration unknown). Death may have been due to mites, or to the condition of the duodenum (cause unknown), or both.

(9) April 2, Rhode Island Red cock.

Right kidney greatly enlarged by tumorous growth (as large as a tennis ball), and duodenum highly inflamed. Heavy infestation of *L. sylviarum*. Death probably due to the other conditions.

(10) July 18, Barred Plymouth Rock cockerel.

Infested with *Capillaria* spp.

Infestation of *L. sylviarum* only fairly heavy. Death probably not due to mites.

(11) July 22, White Plymouth Rock cockerel.

Infested with *Capillaria* spp.

Infestation of *L. sylviarum* very heavy. Death might be due to this, or to *Capillaria* spp., or both.

(12) Aug. 15, Barred Plymouth Rock cockerel.

Tracheae blocked with mucus—bronchial trouble from March 7.

Infestation of *L. sylviarum* very heavy from March 26. Death probably due to the bronchial trouble, as the bird was sickly before infestation.

(13) Oct. 21, Barred Plymouth Rock cockerel.

*Ascaridea galli* present but *L. sylviarum* infestation also very heavy from June 15.

Death might be due to either or both *L. sylviarum* and *A. galli*.

This bird was outside from Sept. 7 to Oct. 1, but the very heavy infestation remained the same.

Of the above birds, Nos. 9 to 13 had been artificially infested since March 7.

#### *Observations on Live Birds*

Records were kept of the weight, egg production, and infestation of 29 pullets from Feb. 12, 1936, to March 7. Unfortunately, the fowls were also infested with *Capillaria* spp. and infected with bronchitis. Many birds died about the middle of March.

Since so many factors, such as heredity, egg production and general health, are involved besides mite infestation, it is difficult to draw any conclusions from these observations. Therefore, the writer cannot support at present those authors who state that this mite causes great economic loss.

Birds which are in poor health owing to internal parasites, disease, heavy egg production, etc., tend to fall an easy prey to ectoparasites, since they become listless and consequently uncleanly in their habits. Thus the writer feels that mite infestation may be a secondary condition and not the primary cause of lack of condition or ill health.

#### B. MAN

Riley and Johannsen (45) cite an instance of *L. sylviarum* attacking man and causing pruritis. This mite readily leaves infested birds, if the latter

are handled, and goes on to man, as already noted. It has been the writer's experience that this mite readily attacks man and bites soft-skinned parts of the body, causing intense itching.

Sambon (47) suggests that *L. sylviarum* can convey "chicken pox", caused by the filterable virus *Epithelioma contagiosum*, from birds to man. The better name for this disease is "fowl pox", since "chicken pox" is the name usually given to an acute contagious disease (principally of young children) caused by *Varicella* sp. and not infective to poultry. There seems to be no evidence to prove that man may become infected by "fowl pox" caused by *Epithelioma contagiosum*.

Since *L. bursa* and *L. sylviarum* are so closely related, the following reports are of interest. There are records of *L. bursa* biting humans (Cilento (13) ) in an area in Australia where a disease occurs which closely resembles the mite-borne river fever of Japan. From other parts of Australia, from Zanzibar, Africa, India, and China, attacks of *L. bursa* on man are reported (Hirst (22, 23, 25) ), (Roberts (46) ).

Monteiro (40) failed to infect guinea pigs with *Rickettsia brasiliensis* sp.n., by injecting, peritoneally, some crushed specimens of *L. bursa*. Hirst (22) reports one specimen from a lizard, while Roberts (46) says that *L. bursa* attacks any animal after birds have left the nest. In 1935, specimens of *L. sylviarum* were taken from a Labrador collared lemming kept in the Institute of Parasitology at Macdonald College. There is a possibility that *Rickettsia* bodies may be transmitted by *L. sylviarum* and *L. bursa*.

## VII. Control

### HISTORICAL

Wood (59) was the first to report successful control of *L. sylviarum*. He recommends the following procedure.

Clean the poultry plant thoroughly and spray it with carbolineum. In warm weather dip fowls in a mixture of 1 gal. water, 1 oz. soap, and 2 oz. sulphur. (This method was also successful with *Cnemidocoptes gallinae* Raillet.)

In cold weather, dust fowls with sulphur. (Fowls so treated suffered no ill results even when sent out into rain. Half-grown chicks and mother hens were treated similarly. With baby chicks, the brood coops were cleaned and dusted with sulphur.)

Destroy nests of the European sparrow.

Wood also had success by dusting with pyrethrum or dipping in a mixture of 1 gal. water,  $\frac{1}{2}$  oz. soap, and 1 teaspoonful of nicotine sulphate. He found that mercuric ointment greatly reduced the numbers but did not give 100% control. A lime-sulphur solution effectively killed the mites but broke down the feathers very badly.

Troop (51) reported "quite" successful results with sulphur dusting and found that nitro-benzol fumigation controlled mites but tainted unlaied eggs.

In the control of *L. sylviarum*, Bishopp and Wood (7), Kaupp (30), Bushnell and Brandly (9), and Maw (36) all recommend sulphur (dusting or dipping) with carbolineum spraying. However, Cleveland\* (15) did not get complete control by such methods and adds that birds brought from outside or returning from shows should be isolated and inspected. If infested, they should remain in isolation until successfully treated.

Bishopp (5) and Caesar (10) replace carbolineum by anthracene oil, a good grade of kerosene oil, or creosote oil. Caesar adds one part of sodium fluoride to every four parts of sulphur to kill lice. Payne\*(43) also uses sodium fluoride for control of lice.

Payne\* (42, 43) found that the greasing of small chicks with lard and vaseline was effective. For other birds, nicotine sulphate, applied 20 min. before roosting time every few days, was successful at any season and eliminated handling of the fowls.

Cutwright\* (16) achieved temporary control by giving roosts a single treatment of nicotine sulphate and dusting nests with sulphur. Dipping birds and painting and spraying houses with a creosote and kerosene mixture failed to give control.

Bushnell and Brandly (9) state that dipping fowls in  $\frac{1}{2}\%$  zenoleum gives control, but best results are obtained by three treatments of nicotine sulphate on the roosts. Nicotine sulphate is also recommended by Bishopp and Wood (7), Jull (29), and Taylor (50).

Bishopp and Wagner (6) stated that a single treatment of nicotine sulphate is not sufficient, and the number of treatments depends upon the extent of infestation and the supplemental measures employed. After a reading of this paper, Cutwright reported verbally that in 1929 there was a severe infestation of 2,500 birds on the Experimental Farm at Wooster, Ohio, and one application of nicotine on the roosts killed all mites in 24 hr. No reinfestation occurred over a period of two years' observation.

Maw (36) states that nicotine sulphate is not satisfactory in all cases and that too much is detrimental to birds. An oil spray in the plumage of birds, as used in stables or on cattle, has given good results.

Whitehead and Maw (58) recommend a mixture of one part naphthalene flakes to two parts vaseline as giving 100% control on birds when applied around the tail and vent, or on the perches. Repeated tests were made, all of which were satisfactory. A mixture in the same proportions of "dichloricide" (paradichlorobenzene) and vaseline gave satisfactory results, but the "dichloricide" costs more and tends to liquefy the vaseline. Nicotine sulphate was used on perches, but comparative tests in the laboratory indicated that its action is slower than that of naphthalene or dichloricide. Naphthalene and vaseline also controlled lice, but the fumes were more lethal to mites.

Maw (38), in laboratory tests on mites, reported on various commercial tar oil preparations, most of which were highly satisfactory.

## NAPHTHALENE AND DICHLORICIDE SALVES

A solid dichloricide salve may be made for summer use by mixing some paraffin wax with vaseline. The dichloricide is finely powdered and stirred into the molten wax-paraffin mixture and allowed to cool. This salve can be made of any desired consistency by varying the proportions of paraffin wax to vaseline, and will not melt at summer temperatures.

The roosts of two pens were smeared with naphthalene and dichloricide salves respectively. About a dozen male birds, infested with *L. sylviarum*, were placed in each pen. Paper was placed beneath the roosts. One day later, some inactive mites were found on the paper. However, there was scarcely any appreciable decrease in the infestation on the birds in either pen, although most of the mites had migrated from the tail feathers to beneath the wings and along the back. No further effects were noted during the next week, and the mites again returned to infest the anal feathers. Naphthalene and dichloricide, in mixtures of twice the strength recommended by Maw (38) for application to the bird's body are relatively ineffective when used on the roosts.

## NICOTINE SULPHATE ON BIRD'S BODY

On March 12, 1936, 12 White Plymouth Rock laying pullets were treated with nicotine sulphate by applying drops to the anal feathers, under each leg, under each wing, and on the neck. On March 15, these birds were clean, while untreated infested birds (controls) in the pen still carried mites. No injury was done to the birds, but this method of control involves handling.

## COMPARISON OF "DICHLORICIDE", NAPHTHALENE, AND NICOTINE SULPHATE

Six male and three female birds, heavily infested with mites, were placed individually in coops. The latter were divided into three groups, and had the roosts smeared with either naphthalene salve, dichloricide salve, or nicotine sulphate liquid (Black Leaf 40). The birds were examined daily for seven days. At that time, the infestation was unchanged in the first two groups, as were three controls in untreated coops. But the three birds treated with nicotine sulphate were clean, the female being free of mites from the second day, the males from the fourth and fifth days, respectively. Attempts to reinfest these birds on the sixth and seventh days were unsuccessful.

Eggs laid by hens in the first two groups were slightly tainted by the chemicals used in the salves.

## FURTHER TESTS WITH NICOTINE SULPHATE

The roosts in a pen housing 55 infested birds (White Plymouth Rock laying pullets) were treated with  $1\frac{1}{2}$  oz. nicotine sulphate. This was spread over the entire 60 ft. of roost space. The following evening a further  $\frac{1}{2}$  oz. was applied to roosts and edges of nest boxes. All birds found off the roosts were put on them. Five of the birds had been placed in coops above the nest

boxes, as controls, on the first day. Five days later, these controls carried their original infestation, while the remaining 50 birds were free from mites. The controls were freed by smearing drops of the liquid on tail, thighs, wings, and head. They showed no ill effects.

All of 57 Barred Plymouth Rock laying pullets, treated in the same way, were free from mites after four days. Four controls remained infested as on the first day. They were treated as above.

Nicotine sulphate was used in several other infestations, and in each the birds were completely freed of mites by a single application. One may conclude, therefore, that nicotine sulphate, when applied under the above conditions, will control the northern fowl mite, *Liponyssus sylviarum*, C. and F. A single application of 2 oz. to every 50 birds is sufficient. The cost is about  $\frac{1}{4}$ c. per bird.

### Recommendations

Infested birds should be isolated from all others and should not have the same attendant as the clean birds.

In the infested pens, during frosty weather, the roosts, dropping boards and nest boxes should be scraped clean; and, about 20 min. before the birds go to roost, the roosts, roost supports and lower front edge of each nest box should be smeared with nicotine sulphate. A bird entering a nest box comes in contact with the nicotine sulphate on the front edge and is soon rid of mites. With a small brush apply about 2 oz. for every 50 birds present and treat every infested pen on the same evening, making sure, if possible, that every bird goes to roost.

Every bird arriving from another farm, plant, show or other place, should be isolated, examined for mites, treated (if infested) and kept in isolation until completely rid of the pest.

Single birds may be hand-treated with a few drops of nicotine sulphate smeared around vent, under thighs and wings, and on the neck.

Make a practice of treating all birds as soon as they enter winter quarters in the fall, since they may have picked up infestation from wild birds; and besides, this treatment is effective against lice. Prevent wild birds from nesting near poultry houses.

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## RELATION OF pH TO DRIP FORMATION IN MEAT<sup>1</sup>

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### Abstract

The quantity of drip obtained from meat frozen at a constant rate is affected by the period between slaughter and freezing, and the pH of the tissue. These two factors appear to act independently, and only the latter was studied extensively. In precooled meats the maximum amount of drip was obtained at about pH 5.2, and as the pH increased the net drip decreased to zero at about pH 6.4. Pork, beef and mutton behave similarly both with respect to the form of the drip-pH relation and the quantity of drip exuded at a given acidity. Beef is normally more acid than the other meats tested, and this can account for its greater tendency to drip in commercial practice.

Microscopic studies showed that large crystals were always produced by slow-freezing, regardless of the pH of the material. The absence of drip from slowly frozen tissue at pH 6.4 is therefore not due to crystal size, but must be attributed to the greater re-absorbing power of the proteins in this region. Protein denaturation does not affect the quantity of drip obtained when meat is slowly frozen or stored for periods up to three days in the freezing zone. The weak re-absorptive power of the proteins at pH 5.2 must be attributed to their isoelectric condition in this region, rather than to their denaturation. It is only in this isoelectric region that the production of small crystals by quick-freezing will reduce the quantity of drip.

### Introduction

This investigation is concerned with the effect of the length of storage period between slaughter and freezing, and the pH of the tissues, on the amount of liquid exuded (drip) from beef, pork and mutton after freezing and thawing. Previous investigations on this subject have been concerned mainly with the effect of freezing and thawing rates on the exudation of drip, and little attention has been given to the condition of the muscle tissue in relation to the amount of drip obtained after freezing. During an investigation of the effect of freezing rate on the quality of poultry (11), it was found that the amount of drip obtained at a given rate of freezing decreased rapidly during the first day after slaughter, and continued to decrease at a much slower rate over a 4-week storage period at 0° C. Other evidence indicating that the condition of the tissues has some effect on the exudation of drip is obtained from the fact that pork and mutton normally do not drip to the same extent as beef when frozen at similar rates.

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Several hypotheses have been offered to explain the cause of drip. Since rapid freezing reduces the size of the ice crystals, and also reduces the amount of drip exuded from such meats as beef, these two observations have been associated and the cause of drip explained in terms of various effects produced by the large ice crystals (3, 10, 12). Others (5, 9) attributed drip to both crystal size and protein denaturation, since it was found that the time and temperature conditions necessary to produce large crystals are also those which produce a rapid rate of denaturation. Most of these hypotheses fail to explain why different meats react differently to the same freezing treatment.

Empey (4) has investigated the drip from beef in relation to the period between slaughter and freezing, and the pH of the tissues, as well as the freezing rate. Contrary to the findings of most other investigators, he reports that rapid freezing does not diminish drip. He also concludes that the period between slaughter and freezing does not influence drip formation. With respect to pH, he found that although the quantity of drip did not bear a definite relation to the pH value of individual muscles, there was a zone in the vicinity of pH 6.3 in which the drip was minimal. Since the reaction of beef muscle changes from about pH 7.0 to pH 5.5 within a day or so after slaughter, his conclusion that the period between slaughter and freezing has no effect on the quantity of drip is difficult to harmonize with his conclusion that the drip is least in the region of pH 6.3.

Although Empey's conclusions do not appear to be entirely consistent, his results indicate that the pH of the tissues has some effect on the exudation of drip. In the investigation on poultry, previously cited (11), an attempt was made to relate the decrease in drip, during storage prior to freezing, to the pH changes in the tissue. The results were inconclusive. Beef, pork and mutton were therefore used in this study to determine the effect of pH on drip exudation, since these meats exhibit free drip to various degrees after similar freezing treatments.

### **Drip in Relation to Storage Before Freezing**

Three experiments of a preliminary nature were made to determine the effect of storage, prior to freezing, on the quantity of drip obtained from beef and pork frozen at a constant rate (4 hr. to pass from 0° C. to -5° C.). The beef used for the first test was obtained from the hind quarters of a 3-year-old heifer, and that for the second test from the hind quarters of a poor quality aged cow. The pork was obtained from the hams of three pigs.

Storage temperatures of 0° C. and 10° C. were used. The meat was placed in rooms at these temperatures within one hour after slaughter. At the desired intervals, 2-lb. pieces of meat were removed, minced, placed in the freezing cans (100 gm.) and frozen. The samples were then stored at -22° C. for several days and then thawed within 4 hr. in an air bath at 15° C. Drip was determined by the blotting paper method previously described (11). Free drip was also determined on these samples after the same storage intervals by freezing approximately 1-lb. pieces, in duplicate, in closed containers and collecting the free liquid exuded on thawing.

The pH of the minced samples was taken before and after freezing, as well as after the drip determination. Twenty-four hours after slaughter, all of these observations gave similar values. The pH measurements were made with a glass electrode, and the values were corrected to 20° C., although the majority of observations were made at 0° C.

In Fig. 1, the total drip from the frozen beef and pork samples, and the drip from the corresponding unfrozen controls, have been plotted against the storage period. The number which appears beside each of the points is

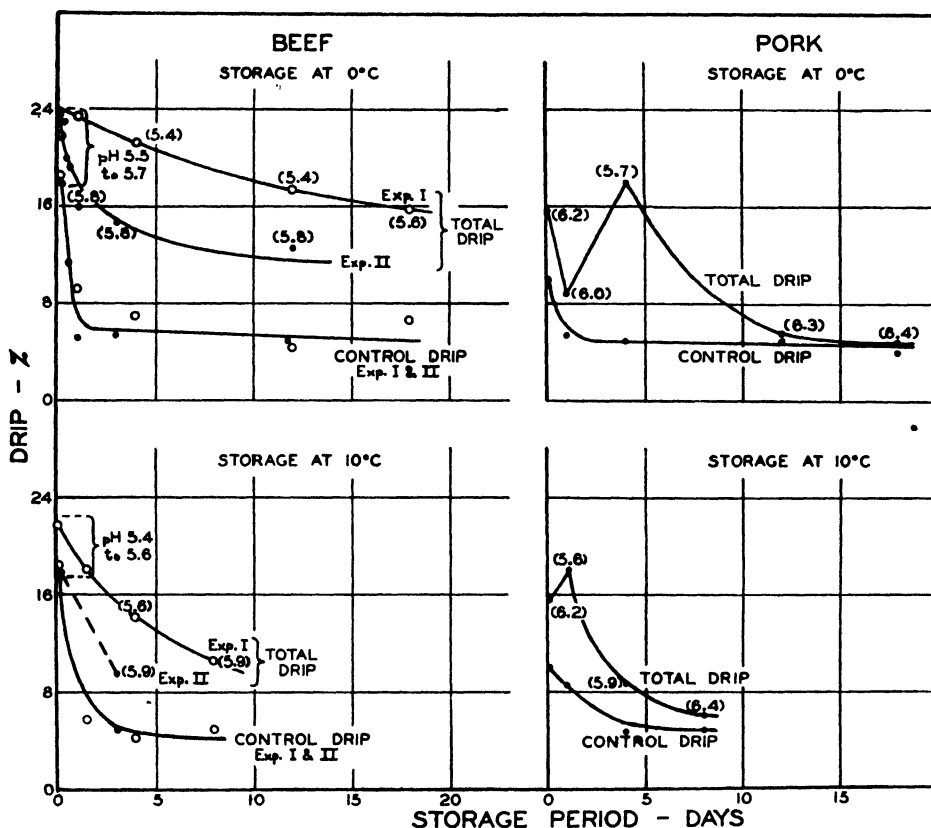


FIG. 1. Drip in relation to storage before freezing.

the pH of the sample at the time of thawing. It is evident that the drip from the unfrozen (control) samples of beef decreases markedly during the first day or two after slaughter, after which it remains relatively constant. The total drip from frozen beef decreases slowly throughout the storage period at 0° C. and more rapidly at 10° C. The pH of beef had usually fallen to a minimum value before the first drip determinations were completed, and remained relatively constant throughout the storage period at 0° C., but increased somewhat at 10° C.

The drip from the unfrozen pork samples behaved in a manner similar to beef. The total drip from the frozen samples, however, passed through a

more erratic sequence of changes during storage. In contrast to the relatively constant pH values observed in beef, the pH of the pork samples at first decreased, and then increased markedly during storage. These variations in total drip and pH may be the result of a difference between the meat from the three pigs used in this experiment rather than a true storage effect.

The results in Fig. 1 suggest that the quantity of drip obtained from properly precooled material is related to the pH of the tissue at the time of freezing. Thus the beef in the first test, having a pH of 5.4, yielded more drip after freezing than the beef at pH 5.8, used in the second test. The results with pork also indicate that the maximum total drip is associated with low pH values.

### Significance of Free and Net Drip Determinations

It is appropriate to discuss here the significance of the results obtained by the two alternative methods employed for determining drip. Free drip is usually determined by freezing pieces of whole muscle tissue and determining the drainage on thawing. The net drip is obtained by determining the amount of liquid expressed, by some adopted procedure, from both the frozen (total) and unfrozen (control) material, the difference between these two quantities being the net drip attributable to freezing. In conjunction with the experiment discussed in the previous section, an attempt was made to determine the relation between these two quantities. The variable nature of the few data obtained precluded a definite conclusion, and the results are not presented. The observed variations can be attributed primarily to differences in the area of cut surface, differences between different muscles (4), and to different pH values in the whole and minced material. Where these factors have been controlled (7), the net and free drip appear to be in good agreement. The results obtained in this study, although variable, substantiated this finding for pork, and for beef frozen within 24 hr. of slaughter. From beef stored for longer periods, the net drip obtained was about 5% (in terms of the meat) higher than the free drip.

Since the net drip procedure employed with minced tissue gave much more reproducible results than the free drip determination, it was adopted for subsequent experiments. It is doubtful if the results obtained by this method provide a quantitative estimate of the free drip in meats subjected to different storage and freezing treatments. It has, however, the added advantage of showing the effect of a given treatment on the moisture-retaining capacity of both the unfrozen and frozen tissue.

In Fig. 1 the drip from both the frozen and unfrozen tissues has been plotted against the storage period instead of the net drip. It is evident that some change occurs in the unfrozen tissue immediately after slaughter, and that this markedly decreases the quantity of water that can be removed as drip. As the drip from the frozen material decreased comparatively slowly throughout the entire prefreezing storage period, it would appear that these rigor or post-rigor changes affect the moisture retention of the unfrozen

material only. If this is so, then the practice of computing the net drip by difference is unsatisfactory for meats frozen within 24 hr. after slaughter, since it combines the effect of these changes in the unfrozen sample with the effect of the freezing treatment on the frozen sample.

It appeared from these preliminary experiments that the factors determining the water-retaining ability of both unfrozen and frozen muscle tissue merit investigation. Since the method employed for determining drip was inadequate for a critical study of changes that occur within 24 hr. after slaughter, this phase was abandoned in favor of investigating the factors that affect the quantity of drip obtained when aged material is frozen.

### Relation Between Drip and pH

Since the preliminary experiments indicated that the amount of drip (from material that had been properly precooled before freezing) was dependent on the pH of the tissues, this subject was the next to be investigated. The results of the experiments described above and those of other investigators (2, 6), indicate that the pH of beef, a few days after slaughter, is relatively constant, whereas the pH of pork is subject to some variability. For this reason pork was used.

The meat was obtained from carcasses that had been cooled or stored for at least 48 hr. after slaughter. Such material varied in pH from a minimum of 5.6 to a maximum of 6.3, but the majority of the samples fell within a much narrower pH range.

In order to extend this range, and also to obtain a more uniform distribution of samples within it, an attempt was made to adjust the acidity by artificial means. The method employed was to inject the estimated amount of 85% lactic acid or 26% ammonium hydroxide into 800- to 900-gm. pieces of pork. In all cases the acid or alkali was diluted to a final volume of 20 ml. before being injected at numerous uniformly distributed points with a calibrated 5-ml. hypodermic syringe. In producing a range of pH values by this method, some of the samples did not require adjustment and were injected with 20 ml. of water, to render them comparable with the treated material. Following injection, the samples were stored for 3 days at 0° C. to allow the acid, or alkali, to diffuse. They were then minced, triplicate samples removed, frozen at a constant rate (*i.e.*, 4 hr. to pass from 0° C. to -5° C.) and the same number kept as unfrozen controls. The drip determinations on these samples were then made in the usual way.

Several objections may be raised to this artificial method of preparing the samples. The added water, although small in proportion to the amount present in the muscle, is undesirable. Again, the variable amount of acid or alkali present will cause some variation in the osmotic pressure. Other objections could be detailed, but they could scarcely introduce sufficient bias to be detectable by the method employed for determining drip. This contention was supported by the results obtained. Reference to Fig. 2 shows

that the curves relating drip and pH in normal and treated samples coincide, within experimental error, over the pH range encountered in normal samples.

This method of adjusting the pH was then applied to beef, which normally has a comparatively constant pH that shows little change during storage (Fig. 1). A single series of experiments was also made with mutton. Casual observations made during the course of these experiments indicate that mutton behaves like pork, in that the pH is subject to considerable variation between animals, and probably increases more rapidly during storage.

The observed drip from the frozen (total) and unfrozen (control) samples and the difference between them (net drip) have been plotted against the pH in Fig. 2. It is evident from the curves that these three quantities are related in meats stored two days or more after slaughter. A pH condition which causes an increase in the drip from the unfrozen control also causes an increase in the total and net drip. This relation has previously been reported for poultry meat (11).

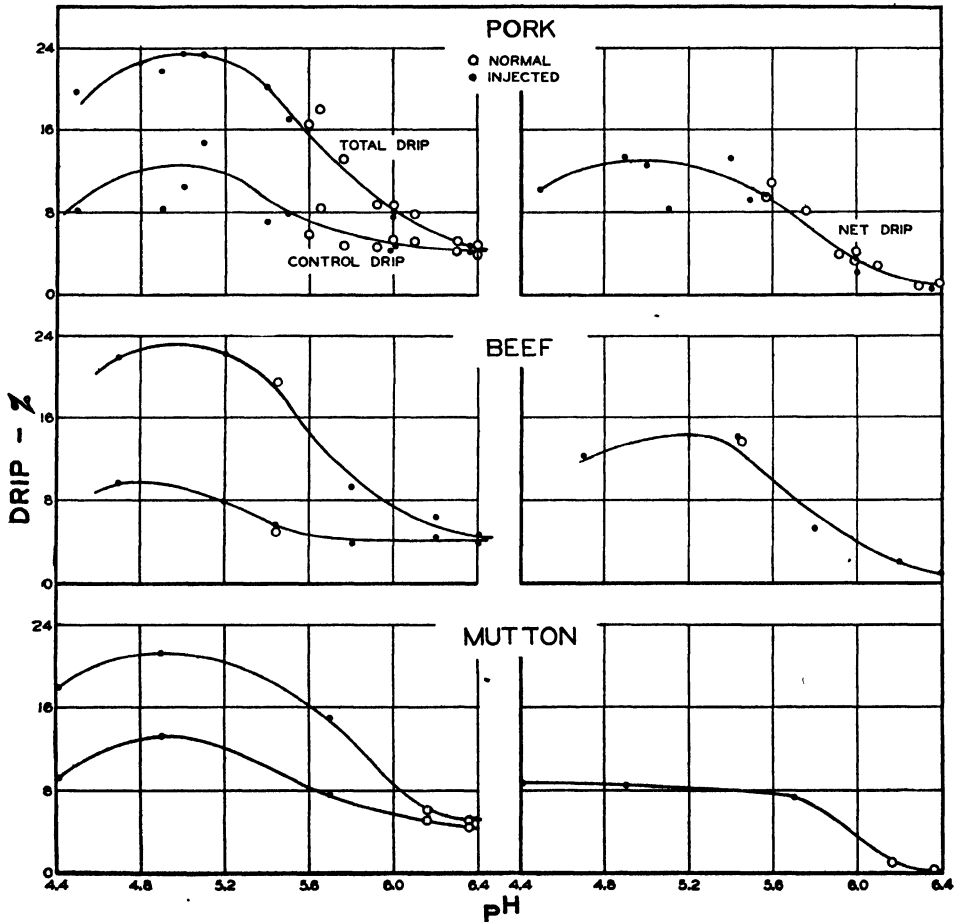


FIG. 2. Drip in relation to pH of muscle tissue.

The curves in Fig. 2 show a marked relation between the amount of drip and the pH; all three meats showing maximum drip between pH 5.0 and 5.2, the curves descending slightly to pH 4.4, the lowest value tested. On the alkaline side of pH 5.2, the drip decreases markedly to about pH 6.4, where the effect of freezing cannot be detected, *i.e.*, net drip is zero. A few samples having pH values between 7.0 and 9.0 were tested, and there was no evidence that the drip increased within this region.

Although the shape of the total drip-pH curves for all three meats is identical, within experimental error, the quantity of drip obtained from the unfrozen samples of beef was somewhat less than that obtained from similar samples of pork and mutton. The result is reflected in a somewhat higher net drip for beef, which is of questionable significance, since it was mentioned in an earlier section that the method used for determining net drip probably overestimates the free drip from beef by about 5% in stored material such as that used in these tests. It is therefore concluded that these three meats will drip to the same extent after freezing and thawing, provided that they have the same pH and that the same freezing rate is used. The experience of practical operators and the results of investigation (3, 12), show that more drip is obtained on thawing slowly-frozen beef, than on thawing slowly-frozen pork or mutton. This behavior can be attributed to the pH values typical of these meats. The pH of beef is relatively constant and close to the value at which maximum drip occurs, whereas pork and mutton vary in pH from carcass to carcass, are generally more alkaline, and tend to become more so during storage. This fact can readily explain the small amount, or practical absence, of drip from frozen pork or mutton.

### Relation Between Crystal Size and pH

The exudation of drip from meat after freezing and thawing is frequently attributed to various effects produced by the formation of large ice crystals during slow-freezing. As shown in the previous section, meat at pH 6.4 does not drip even after slow-freezing. In order to fit this fact into the hypothesis that crystal size determines the formation of drip, it is necessary to assume that pH affects the size of crystal formed. This assumption is not untenable, since the necessary condition for the formation of large ice crystals is that the rate of movement of water from the tissue to the existing ice nuclei during freezing exceed the rate of crystal formation as determined by the cooling rate. Since the acidity of the tissue may affect the rate of moisture movement, the effect of pH on the crystal size at various rates of freezing was investigated.

Several samples of pork and beef were adjusted to pH values varying from 5.5 to 6.5 by the procedure already described. These samples were passed through the freezing zone (0° to -5° C.) in one and in 24 hr. to produce quick- and slow-frozen material respectively. The samples were then examined microscopically with both reflected and transmitted light using material in the frozen and fixed (7) condition. Numerous examinations showed that the crystal size depended entirely on the rate of freezing, any slight effect of

pH being indistinguishable from the variations between sections of the same material. This is illustrated in Plate I, which shows typical photographs of fixed material (approx. 5 $\times$ ), quick- and slow-frozen at pH 5.5 and 6.5. It is evident from these results that crystal size alone is not an adequate hypothesis for explaining the formation of drip.

### **Effect of Freezing and Storage at Different pH Levels on Protein Solubility**

Since the foregoing experiments indicate that drip is markedly influenced by the pH of the tissues, while crystal size and the structural differences between the three meats tested are of secondary importance, it would appear that the condition of the proteins must be the primary factor responsible for the retention of fluids by muscle tissue after freezing.

The fact that the maximum drip occurred in the region of pH 5.2 could be attributed to either the isoelectric condition of the proteins or to their more rapid denaturation in this region. Bate Smith (1) has shown that myosin and globulin X constitute 89% of the muscle proteins, and that their isoelectric points are pH 5.5 and 5.2 respectively. In support of the second possibility, Finn (5) has shown that denaturation (as indicated by the amount of protein precipitated from muscle juice during 24 days' storage at  $-2^{\circ}$  to  $-3^{\circ}$  C.) occurs readily on the acid side of pH 6.0. Certain investigators (8, 9) consider that denaturation is at least partly responsible for the exudation of fluids after freezing, storage, and thawing. Since the isoelectric point is generally the region of minimum colloidal stability, it would appear that loss of solubility, or water-retaining capacity at this point, does not necessarily indicate denaturation, or an irreversible alteration of the protein. Unfortunately this is difficult to prove since denaturation cannot be defined except in relation to the method used for its detection.

In the first experiments an attempt was made to relate the quantity of drip to the amount of nitrogen extracted by 1.2 molar potassium chloride, buffered at pH 7.2 as recommended by Reay (10). Meat samples at different pH levels were frozen at a constant rate (4 hr. to pass from  $0^{\circ}$  C. to  $-5^{\circ}$  C.) and stored at  $-22^{\circ}$  C. for several weeks. Another experiment, using a different sample of pork, was performed by storing the material at  $-3^{\circ}$  C. for 12 days, since the rate of denaturation is reported (5, 8, 9) to be most rapid in this region.

The results appear in Table I. The pH values marked by an asterisk were obtained after the injection of ammonium hydroxide. The quantity added was equivalent to about 0.3% in terms of the extractable nitrogen, and this amount was therefore subtracted in order to obtain the reported values. The other samples had attained the reported pH values naturally. It is obvious that, although the pH and the quantity of drip varied markedly, the quantity of nitrogen extracted remained relatively constant. Similarly the amount of nitrogen extracted from the samples stored at  $-3^{\circ}$  C. was quite constant and hence showed no evidence of denaturation. On the other hand

TABLE I  
COMPARISON OF *E. revolutum* FROM THE GOOSE WITH THAT FROM THE DUCK

—	Body	Collar spines	Cuticular spines	Oral sucker	Pharynx	Oesophagus	Acetabulum	Testes	Vitellaria	Ovary	Egg
Specimens from goose	Elongate	No. = 37	Numerous anteriorly. None in post-testicular region	Sub-terminal	Muscular	Long, slender	2.6 mm. from anterior end. Sub-globular	Oval, entire margin. Lengthwise of body	From posterior of acetabulum to posterior extremity	Equatorial in position, pear-shaped	Oval
	15.9 × 2.57 mm.	70-15' μ × 30-32 μ		0.40 × 0.25 mm.	0.29 × 0.34 mm.	1.06 mm. long	1.31 × 1.36 mm.	Ant. 1.26 × 0.84 mm. Post. 1.42 × 0.87 mm.		0.54 × 0.37 mm.	100-110 μ × 52-60 μ
Specimens from duck	More or less flask-shaped	No. = 37	Numerous anteriorly. None in post-testicular region	Sub-terminal	Muscular	Long, slender	1.46 mm. from anterior end. Sub-globular	Oval, lobulated. Crosswise of body	From posterior of acetabulum to posterior extremity. Distinct follicles	Equatorial in position, lobulated oval	Oval
	6.65 × 1.94 mm.	70-80 μ × 17-18 μ		0.24 × 0.22 mm.	0.17 × 0.22 mm.	0.49 mm. long	0.69 × 0.64 mm.	Ant. 0.63 × 0.25 mm. Post. 0.65 × 0.27 mm.		0.47 × 0.22 mm.	93-110 μ × 50-54 μ

by 0.137–0.16 mm. The thin-walled cirrus-sac, 0.2–0.21 by 0.12–0.122 mm., enclosing a powerful cirrus and an internal seminal vesicle, lies dorsal to the acetabulum and extends from a short distance anterior to the anterior margin of the acetabulum, posteriorly to approximately the centre of it. The common genital pore opens ventrally at a point about midway between the caecal bifurcation and the anterior margin of the ventral sucker.

The roundish ovary, 0.09–0.11 mm. in diameter, lies a short distance in front of the anterior testis and slightly to the left of the mid-line. It is separated from the anterior testis by a diffuse shell gland and a vitelline receptacle. The uterus extends from the oviduct to the genital opening, making few transverse loops and containing few eggs. The eggs are comparatively large, measuring 87–91 $\mu$  by 45–49 $\mu$ . The vitellaria occupy the lateral fields of the body and surround the intestinal caeca except on the inner side. They consist of separate groups of small follicles, which do not meet in the mid-line posteriorly. They extend from a short distance caudad of the ventral sucker to almost the posterior end of the worm.

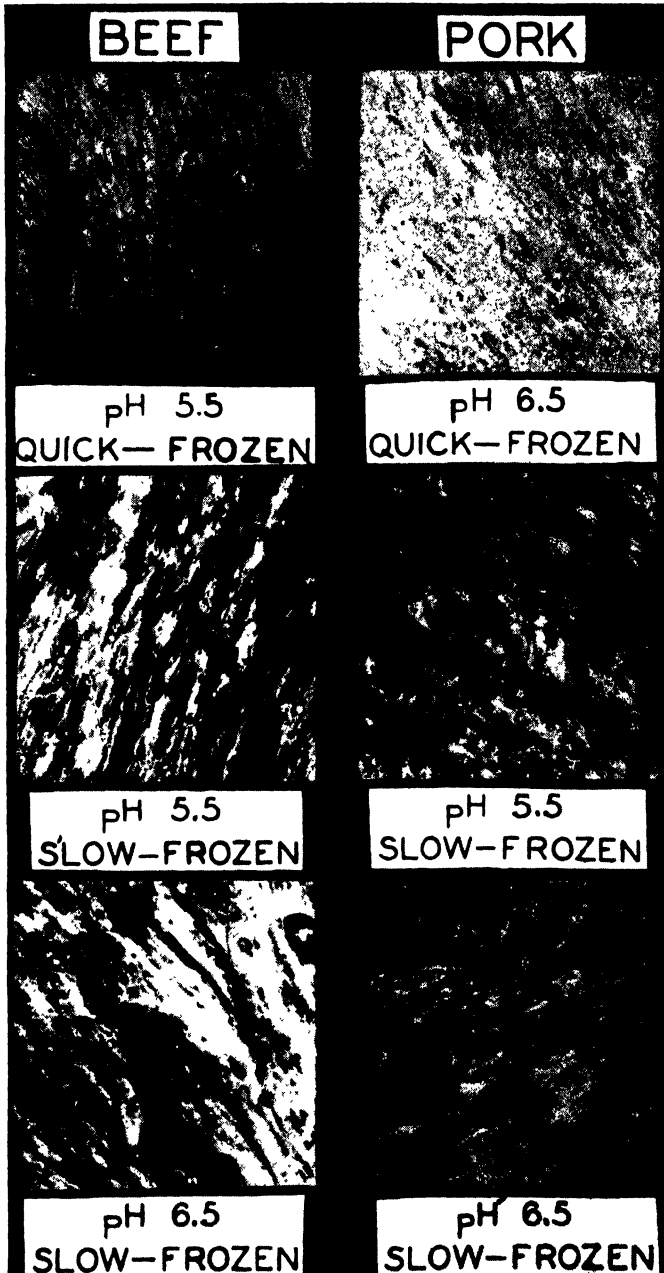
This is the first time that *Echinoparyphium elegans* has been reported from the Black duck, and the author has been unable to find any previous records of *E. elegans* from North America.

*Stephanoprora mergi* sp. nov.

Three small flukes were found in the caeca of an American Merganser duck, *Mergus merganser americanus*. The worms were preserved in ethyl-glycoformol mixture, stained in alum-carmin, cleared in oil of wintergreen and mounted in Canada balsam.

The worm (Fig. 4) is elongate-oval in shape with an almost circular cross section. The neck region, extending from the head collar to the point of bifurcation of the intestine, is only slightly narrower than the posterior portion. The average specimen measures 1.16 mm. long with a maximum width, in the anterior testicular region, of 0.277 mm. A well-differentiated, reniform head-collar is present, measuring 0.17 by 0.19 mm. The collar bears one row of spines that is discontinuous dorsally. In each end group of spines there are two "Echstacheln" and in each lateral group nine "Randstacheln", making a complement of 22 collar spines that vary in size from 25–37 $\mu$  by 5–6 $\mu$ . From the head collar to approximately the anterior border of the anterior testis, the cuticle is provided with fine, closely set, backwardly directed spines which are very numerous anteriorly, becoming more sparse posteriorly.

The oral sucker, measuring 0.053–0.058 by 0.043–0.047 mm., is slightly sub-terminal and appears somewhat drawn out posteriorly. A comparatively long prepharynx is present, measuring 0.061 mm. in one specimen and 0.08 mm. in another. The pharynx varies from 0.072–0.078 mm. in length by 0.05–0.06 mm. in width. It is followed by a long oesophagus that is 0.18–0.2 mm. in length. The intestinal caeca pass postero-laterally and reach almost to the posterior end of the body. The ventral sucker, 0.072–



*Effect of pH and freezing rate on crystal size. 5X.*



the quantity of drip obtained from the stored sample did increase somewhat over that obtained from the corresponding sample representative of freezing alone. This suggests that some change occurred during storage at  $-3^{\circ}\text{C}$ . but that the extraction technique did not detect it.

TABLE I  
EFFECT OF pH, FREEZING, AND STORAGE ON PROTEIN SOLUBILITY

Treatment	Meat	pH	Total drip, %	Extracted nitrogen, %
Freezing only (stored at $-22^{\circ}\text{C}$ .)	Beef	5.2	22.2	1.20
	Beef	6.4*	4.8	1.14
	Pork	5.4	20.4	1.18
	Pork	6.0	7.0	1.24
	Mutton	5.7	14.9	1.26
	Mutton	6.2	6.0	1.22
Freezing only (stored at $-22^{\circ}\text{C}$ .)	Pork	5.5	21.9	1.06
	Pork	6.2*	8.5	1.04
Freezing + 12 days storage at $-3^{\circ}\text{C}$ .	Pork	5.5	28.2	1.08
	Pork	6.2*	9.3	1.05

\* Ammonium hydroxide injections necessary to attain these levels.

It would appear that an extraction procedure is not suited to a study of the nature of the changes in the proteins affecting drip. In the first place, only a fraction of the total protein is extracted, and any irreversible change that occurs at different pH values might take place in the unextracted portion. Secondly, such a method could scarcely yield an estimate of the effect of pH on a reversible colloidal condition, since the amount extracted would be determined by the pH of the extraction medium rather than by the pH at which freezing and thawing took place.

The use of expressed muscle juice for a study of the effect of freezing on the proteins also appears unsuitable. The several muscle proteins are not present in nearly the same proportions in the expressed juice (5), and the pH would require adjustment after freezing and thawing, in order to distinguish between colloidal instability at the isoelectric point and denaturation.

It is concluded from these experiments that the large difference in the quantity of drip obtained at the two pH levels used cannot be explained in terms of denaturation as evidenced by the constant amount of protein nitrogen extracted from all samples. However, since an extraction procedure appears to be unsuitable for a study of the nature of the pH-drip relation, further experiments of this sort were abandoned.

### Contribution of Isoelectric Condition and Denaturation to Drip

Since methods based on the solubility of the proteins appear to be unsuitable for studying the effect of pH on drip, an attempt was made to use methods

based on the reversibility of the pH-drip relation in muscle tissue. The loss of moisture-retaining capacity at pH 5.2 may be attributed to either the isoelectric condition of the proteins (1) or to their denaturation in this acid condition (5). If the first of these factors is the cause of drip, the moisture-retaining capacity should be restored by changing the pH. On the other hand, if denaturation is the cause of drip, it might reasonably be expected to be irreversible when the pH is altered over a limited range.

The experiments undertaken included a study of the effect of freezing and storage at  $-3^{\circ}\text{C}$ . for various intervals. This storage temperature was chosen for reasons already given. A large sample of pork was adjusted to pH 5.4–5.5 by the injection technique already described. After standing two days, the meat was minced, mixed, transferred to the freezing cans, and frozen at the usual rate ( $0^{\circ}\text{C}$ . to  $-5^{\circ}\text{C}$ . in 4 hr.). Four series of 12 samples were prepared in this way. One series was stored at  $-22^{\circ}\text{C}$ ., and the remaining three were held at  $-3^{\circ}\text{C}$ . for 3, 6, and 12 days, respectively, before transferring them to a temperature of  $-22^{\circ}\text{C}$ . until required.

After thawing, one set of triplicate samples from each series was kept at the original pH, and the pH of the other three sets was adjusted to pH 5.7, 6.0 and 6.4, as nearly as possible, by the addition of small increments of ammonium hydroxide. Each sample was then well mixed, stored at  $0^{\circ}\text{C}$ . for 4 days to allow the alkali to diffuse, refrozen, thawed, and the quantity of drip determined.

A similar piece of meat at pH 6.2–6.3 was minced, frozen, stored and treated as described above, except that lactic acid was used to obtain the required pH values.

The results obtained appear as two charts in Fig. 3. The one on the left was constructed from the data obtained from the samples that were frozen and stored at pH 5.4–5.5, and the one on the right from the data obtained from samples stored at pH 6.2–6.3. In both charts the total drip is plotted against the pH after the final adjustment, the value at which the drip determinations were made.

The lower heavy line in both charts is a reproduction of the curve relating total drip and pH, taken from Fig. 2. It is evident that the samples which were not stored at  $-3^{\circ}\text{C}$ ., and those stored at this temperature for only 3 days, fit this curve within experimental error. All of the samples stored for 6 or 12 days at  $-3^{\circ}\text{C}$ ., at either pH 5.3–5.4 or pH 6.2–6.3, yielded more drip than the material stored for shorter periods. The broken line in the two charts (Fig. 3) has been drawn through the points representing the data obtained with this material.

It appears, therefore, that the high total drip observed throughout at pH 5.2–5.4 can be completely reversed by adjusting the pH as long as the storage period in the zone of crystal formation does not exceed 3 days. Similarly, the samples stored at pH 6.2–6.3, which do not yield any net drip, showed the same amount of drip as the samples stored initially at 5.4–5.5, when adjusted to the same pH values, if stored for less than 3 days at  $-3^{\circ}\text{C}$ .

Since the effect of pH on drip is reversible under these conditions, it is concluded that the high drip observed at pH 5.2–5.4 is due to the isoelectric condition of the principal muscle proteins, and not to denaturation.

Although no evidence of denaturation was indicated by the drip determination up to 3 days' storage at  $-3^{\circ}\text{C}$ ., it is evident from the broken line that the pH-drip curve was displaced upwards in material stored for 6 or 12 days at  $-3^{\circ}\text{C}$ . This increase in drip indicates that denaturation did occur over these longer storage periods, although the slope of the curve still indicates a certain degree of reversibility. Since the points obtained after 6 and 12 days' storage fall on the same curve, within experimental error, it

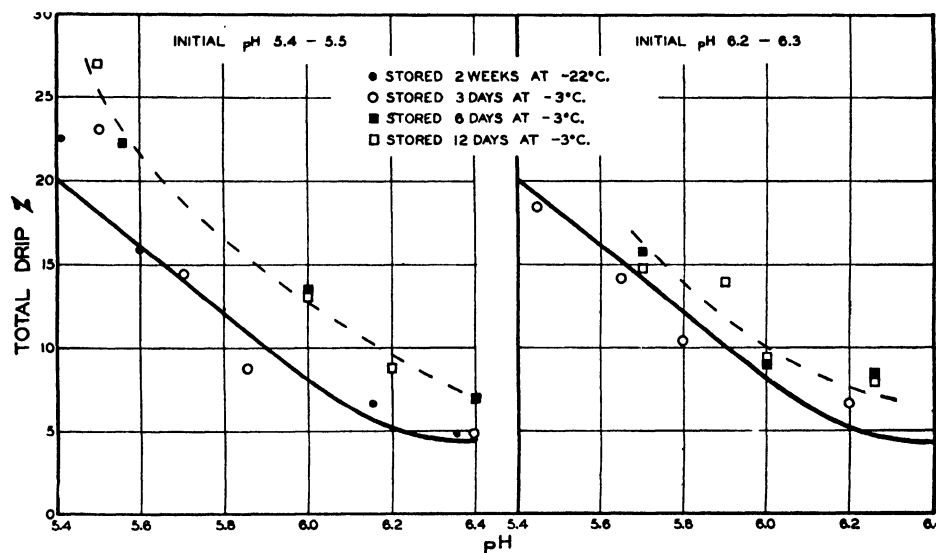


FIG. 3. Reversibility of pH-drip relation in muscle tissue. Solid line, unstored material; broken line, material stored for 6 or 12 days at  $-3^{\circ}\text{C}$ .

appears that denaturation, as it affects drip, is complete in about 6 days. The displacement of the drip-pH curve from that of unstored material is less at pH 6.2–6.3 than at pH 5.4–5.5, indicating that denaturation is more rapid or affects more of the protein in the more acid material. This confirms Finn's results (5).

Since even slowly frozen material would generally pass through the freezing zone in less than 3 days, it appears that protein denaturation is not the factor causing drip under ordinary conditions. Moreover, although the extent of denaturation is greater at pH 5.2–5.4 than at pH 6.3, this difference is small compared with the direct effect of pH on drip. It is therefore concluded that the observed dependence of drip on pH is due primarily to the isoelectric condition of the muscle proteins rather than to their denaturation.

#### Relation of pH to Change of Weight in Salt Solutions

Further evidence indicating that a portion of the water present in muscle tissue is held less firmly at pH 5.5 than at higher pH values was obtained by

observing the changes in weight of pork placed in dry salt or saturated salt solutions. These preliminary experiments were made with 100-gm. pieces of pork, varying in pH from 5.0 to 6.4, by immersing them in saturated sodium chloride solution, or covering them with dry salt for periods up to 10 days, in a room at 5° C.

Reference to the results given in Table II shows that the change in weight is markedly influenced by pH. The samples having a pH of 5.5 underwent no appreciable change in weight in the salt solution, but lost 32.9% of their weight in the dry salt. Samples having a pH of 6.4 gained 15.3% in weight in the salt solution, and lost only 18.6% of their weight in the dry salt. It is evident that pork tends to lose more moisture at pH 5.5 than at 6.4, a result in agreement with the observations made on drip.

TABLE II  
EFFECT OF PH ON WEIGHT AND SALT UPTAKE OF PORK DURING PICKLING

Pickling method	pH of meat	Change in weight in			Salt content of pickled meat, %
		5 hr., %	24 hr., %	10 days, %	
Saturated salt solution	5.0	-3.2	-2.9	-2.7	17.3
	5.5	+0.2	+0.9	+0.1	17.2
	6.0	-0.7	+0.8	+0.8	17.4
	6.4	+2.8	+6.3	+15.3	17.0
Dry salt	5.0	—	—	-34.0	15.8
	5.5	—	—	-32.9	16.3
	6.0	—	—	-19.2	16.0
	6.4	—	—	-18.6	17.2

The above results are preliminary to a more extensive investigation dealing with the effect of pH on salt penetration. Callow (2) has found that salt penetrates acid samples (pH 5.5) more rapidly, a finding that may also be related to the isoelectric condition of the proteins.

### Relation of pH to the Color of Meats

In the last investigation it was noted that the color of the salt solutions was influenced by the pH of the pork. The samples at pH 5.0 yielded a colorless solution, while the alkaline samples (pH 6.4) yielded a deep red solution. Intermediate pH values showed a gradation in color between these two extremes.

It was also observed that the color of meat itself was markedly affected by pH. As indicated previously, an injection method was used to obtain the desired pH values, and it was found that at pH 5.0 the samples became a grayish color, while those at pH 6.5 were a deep red. Intermediate pH values yielded a gradation between these extremes. On readjusting the pH of the acid samples (5.0) to a more alkaline state, the color of the meat changed from gray to red. Undoubtedly some relation exists between pH

and meat color. This was evident for pork, beef and mutton, and this relation is now being studied quantitatively in these laboratories.

### Discussion

The effect of storage, between the times of slaughter and freezing, on the amount of fluid exuded by meat after freezing, could not be determined precisely by the methods employed. It appears, however, that two distinct phenomena are involved. The drip obtained from meat frozen at a constant rate is determined primarily by the amount of acid contained in the tissues, and it increases rapidly as the pH falls below 6.0. In unfrozen material the amount of fluid exuded decreases rapidly during the first day or two after slaughter, regardless of the pH changes. During this period, the difference between the amounts of fluid exuded by frozen and unfrozen material cannot be attributed entirely to the effect of freezing.

After several days' storage, the quantity of drip obtained from both frozen and unfrozen meats appears to be determined by the same factors, inasmuch as an increased drip from unfrozen material is reflected in a similar behavior of the frozen material. Meat at pH 6.4 or higher does not drip as a result of freezing at any rate requiring less than 3 days to pass from 0° C. to -5° C. At pH 5.2-5.5 the amount of drip reaches a maximum, and in this region an increased freezing rate reduces the amount of drip obtained.

This behavior can be explained by a high water-retaining capacity of the tissue proteins at pH 6.4, resulting in the complete retention of the water produced on thawing, regardless of the size of ice crystal formed during freezing. At pH 5.2-5.5, the water-retaining power of the proteins is lower and moisture losses occur. These losses can be reduced by rapid freezing which produces smaller ice crystals, and a more uniform distribution of water when they melt. Under these conditions less fluid is exuded, although moisture can be removed more easily from tissues at these pH values, even if it is not frozen. The reduced moisture-retaining capacity of the tissue proteins at pH 5.2-5.5 appears to be attributable to their isoelectric condition rather than to accelerated denaturation in this region.

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## SOME TREMATODE PARASITES OF DUCKS AND GEESE IN EASTERN CANADA<sup>1</sup>

BY D. G. CANNON<sup>2</sup>

### Abstract

Eight species were found. One, *Stephanoprora mergi*, is described as new, and *Echinoparyphium elegans* and *Psilochasmus longicirratu*s are here recorded for the first time in America. Several species are described from new hosts. The other specimens recorded are *Echinostoma revolutum*, *Hypoderacium conoideum*, *Zygocotyle lunata*, *Apatemon gracilis*, and *Notocotylus attenuatus*.

### Introduction

The recent inauguration of conservation of wild life in North America has focused the attention of zoologists on the breeding grounds of ducks and geese on this continent. In most instances these areas are situated in Canada, and it is here that the problems concerning the health of these birds are important. It is fairly safe to say that the major infestations by internal parasites are contracted in the breeding grounds, owing to the tremendous numbers of birds congregated in a comparatively small area. This is undoubtedly the case when infestations are present in young birds.

Although our knowledge of the trematode parasites of these birds has been considerably increased during late years by many publications in the United States, there is still a lack of information concerning the distribution of species. In order to contribute towards a further knowledge of the internal parasites encountered in ducks and geese, a study was made of the trematode parasites collected in eastern Canada during the general survey work of the Institute of Parasitology.

The scientific names of the birds referred to in this paper are those given by Taverner (14) in "Birds of Canada."

### Description of Parasites

#### *Echinostoma revolutum* (Frölich, 1802)

Five specimens from the small intestine of the domestic goose, *Anser domesticus*, several specimens from the Black duck, *Anas rubripes rubripes*, and three immature worms from the Canada goose, *Branta canadensis*, were examined.

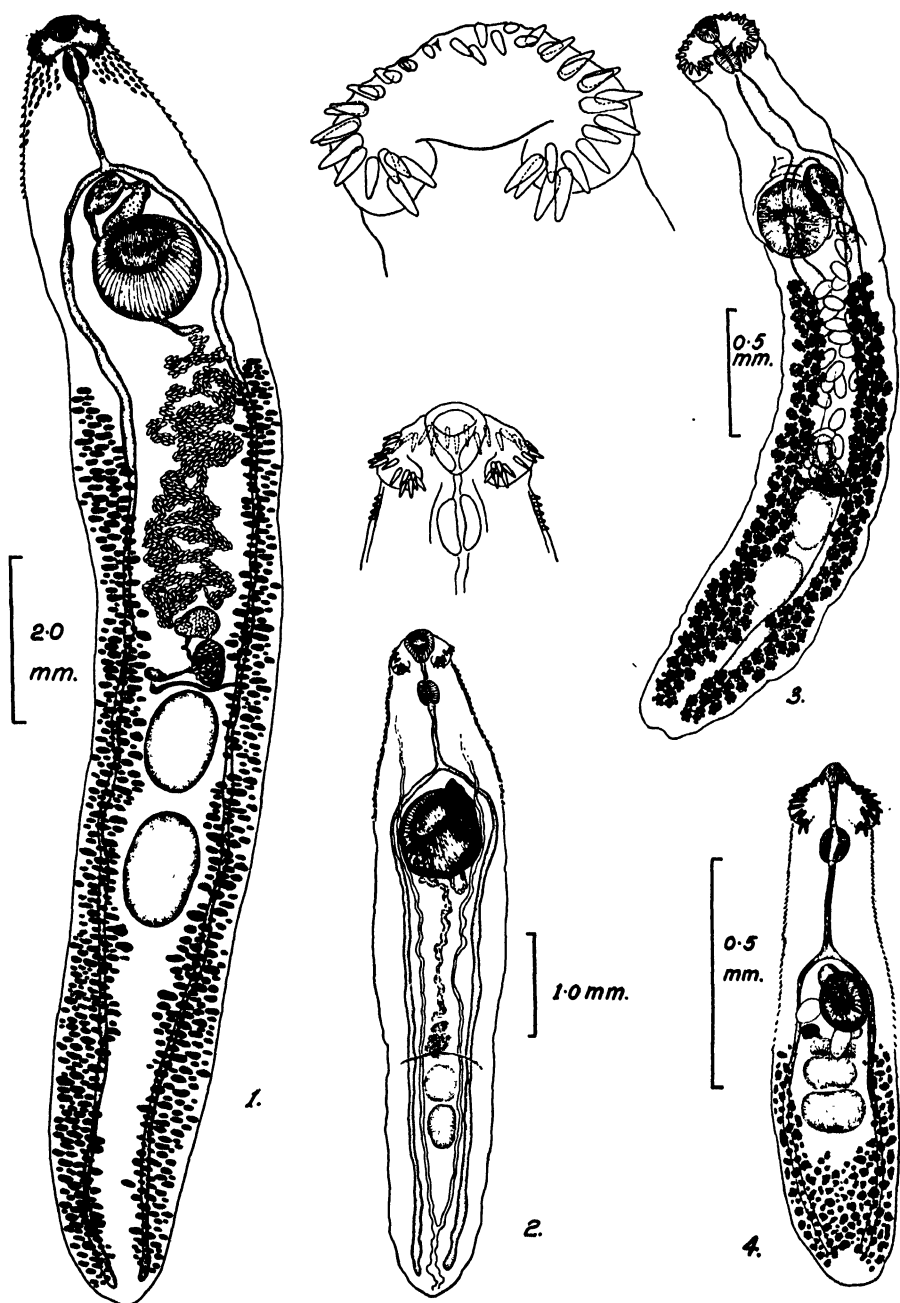
Those from the domestic goose (Fig. 1) were the more typical forms as they adhered closely to the specific description as given by Beaver (1).

The specimens from the Black duck were much smaller; the average worm measures 6.65 mm. in length and 1.94 mm. in width. The shape differs from that of the typical form in that it has a narrow anterior portion which extends posteriorly as far as the acetabulum where it widens considerably, forming

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FIGS. 1-4. 1. *Echinostoma revolutum* (Frölich, 1802), specimen from *Anser domesticus*, entire worm, ventral view. 2. *Echinostoma revolutum* (Frölich, 1802), specimen from *Branta canadensis*, entire worm, ventral view, also magnified anterior end. 3. *Echinoparyphium elegans* (Looss, 1899), entire worm, ventral view, also magnified anterior end. 4. *Stephanoprora mergi* sp. nov., entire worm, ventral view.

shoulders, and gradually narrowing after the uterine region to end in a bluntly rounded posterior extremity (Fig. 2). The collar spines are slightly smaller than those in the typical form, but resemble them in number and position. In Table I the organs of the typical form are compared with those of the specimens from the duck.

The worms from the intestine of the Canada goose were immature. The largest of these is only 3.65 mm. long and 0.73 mm. in breadth at the acetabulum, which is its widest part. In none of these specimens are eggs present. The vitelline follicles could not be distinguished; it is quite possible that they are present, but in their immaturity have not yet taken on their pigment. The 37 collar spines are present and the internal organs of reproduction are situated in the same relative positions as in the typical forms from the domestic goose.

*Echinoparyphium elegans* (Looss, 1899)

Only two specimens were found in the small intestine of a Black duck, *Anas rubripes*, from St. Genevieve, Que.

These are small, slender Echinostomes (Fig. 3), which taper slightly towards the posterior end, with a bluntly rounded termination. The anterior part of the worm has a roundish cross-section whereas the posterior portion is flattened. The ventral sucker appears to be borne on a thick, short stalk so that it protrudes from the ventral surface of the worm. The anterior portion of the worm is bent ventrally. A reniform head-crown is present, bearing a double row of spines which is unbroken dorsally. A full complement of spines could be seen on only one of the specimens, and it bore a total of 42. On each ventral corner lobe of the head-crown is a group of four spines, one pair being superimposed upon the other. The spines of the aboral row are somewhat larger than those of the oral row, the oral spines measuring 40–44.6 $\mu$  by 11.9–13.5 $\mu$ , whereas those of the aboral row measure 50.7–62.93 $\mu$  by 12–16 $\mu$ . The largest spine of the corner groups measures 52.1 by 13.5 $\mu$ . No cuticular spines could be distinguished, but it is quite possible, owing to the roughened and scaly condition of the anterior cuticle, that the spines have been lost as a result of partial digestion of the cuticle by the host. The cuticle of the posterior part of the body did not show this sloughed appearance. The terminal oral sucker measures 0.07–0.08 mm. by 0.087–0.092 mm. There is a short prepharynx. The muscular pharynx is 0.09–0.096 mm. in length by 0.061–0.069 mm. in width. The oesophagus is long and comparatively broad, measuring 0.31–0.36 mm. by 0.061–0.072 mm. The intestine bifurcates a short distance in front of the ventral sucker, and the gut branches terminate blindly 0.115 mm. from the posterior end of the body. The powerful acetabulum, situated about 0.64 mm. from the anterior end of the worm, measures 0.223–0.269 mm. with an opening 0.05–0.058 mm. in diameter.

The testes are elongate-oval in shape and lie median, one behind the other, in the posterior half of the body. The margins are smooth. The anterior testis measures 0.19–0.213 by 0.12–0.14 mm. and the posterior 0.217–0.23

0.130 by 0.04–0.146 mm., is situated with its anterior border about 0.46 mm. from the anterior end of the worm. It is almost equatorial in position.

The two testes are transversely elongate, with entire margins. The anterior, 0.072–0.083 by 0.1–0.15 mm., is slightly smaller than the posterior, 0.084–0.1 by 0.114–0.13 mm. They lie one directly behind the other in the mid-line in the third quarter of the worm. The vasa efferentia and vas deferens could not be distinguished in these specimens. The cirrus-sac is dorsal to the acetabulum and lies somewhat diagonally with its anterior end slightly to the left of the mid-line. It extends posteriorly to the level of the centre of the ventral sucker. The cirrus-sac contains a large *vesicula seminalis*, a small *pars prostatica* and a small cirrus. Both male and female ducts open through a common genital pore which is situated a short distance posterior to the intestinal bifurcation.

The ovary, measuring 0.05 by 0.32 mm., is oval with an entire margin. It lies a short distance anterior to the anterior testis and slightly to the right of the mid-line with almost the whole of its anterior half covered ventrally by the posterior portion of the acetabulum. A uterine seminal receptacle is situated slightly to the left of the mid-line and somewhat posterior to the ovary. A large, diffuse shell-gland is present in the mid-line, lying very close to the anterior testis. Laurer's canal could not be distinguished. The uterus is quite short and forms one loop which extends slightly posterior to the ovary and then bends anteriorly to the genital opening. The ova are 87–90 $\mu$  in length by 40–50 $\mu$  in width. They are very few in number, five being the most present in one worm, and are very large compared with the size of the worm. The vitellaria are composed of large follicles, the individual follicles being situated very close to one another. They extend from the anterior level of the anterior testis almost to the posterior end of the worm. Caudad of the posterior testis the follicles converge towards the mid-line, forming a single mass in the posterior quarter of the worm. The vitelline ducts cross in front of the anterior testis to join and form a vitelline reservoir which occupies a position directly ventral to the shell gland.

*S. merulae* Yamaguti (17) is much larger than the specimen here described. The testes are elongate-oval with their long axes in the mid-line of the worm. The prepharynx is very short and the ventral sucker is more anteriorly placed than in *S. mergi*, whose acetabulum is almost equatorial in position. The cirrus-sac is diagonal but does not extend posterior to the anterior border of the acetabulum.

*S. reynoldi* Bhalerao (2) is also a much larger worm. Its anterior testis is in the anterior half of the body and its posterior testis is in the posterior half, unlike the condition existing in *S. mergi* in which both testes occupy the posterior half of the worm. The vitellaria do not converge until they reach the posterior end of the worm. The ova are somewhat smaller and more numerous. The ventral sucker is more anteriorly placed.

Linton (9) described a fluke which he named *Mesorchis pseudoechinatus*. The genus *Mesorchis* has now been included in the genus *Stephanoprora* (10),

as has also the genus *Monilifer*. The position and shape of the testes of *M. pseudoechinatus* and of *S. mergi* are different. The ova are approximately the same size but are much more numerous. The vitellaria form two dense lateral masses of follicles in the posterior portion of the body. The individual follicle is fairly small.

Several other species of *Stephanoprora* have been described, but *S. mergi* appears to differ from them in its small body size, the shape, size and position of the testes, the more or less equatorial position of the acetabulum, the comparatively long prepharynx and oesophagus, and the extent of the vitellaria.

*Hypoderaeum conoideum* (Bloch, 1782)

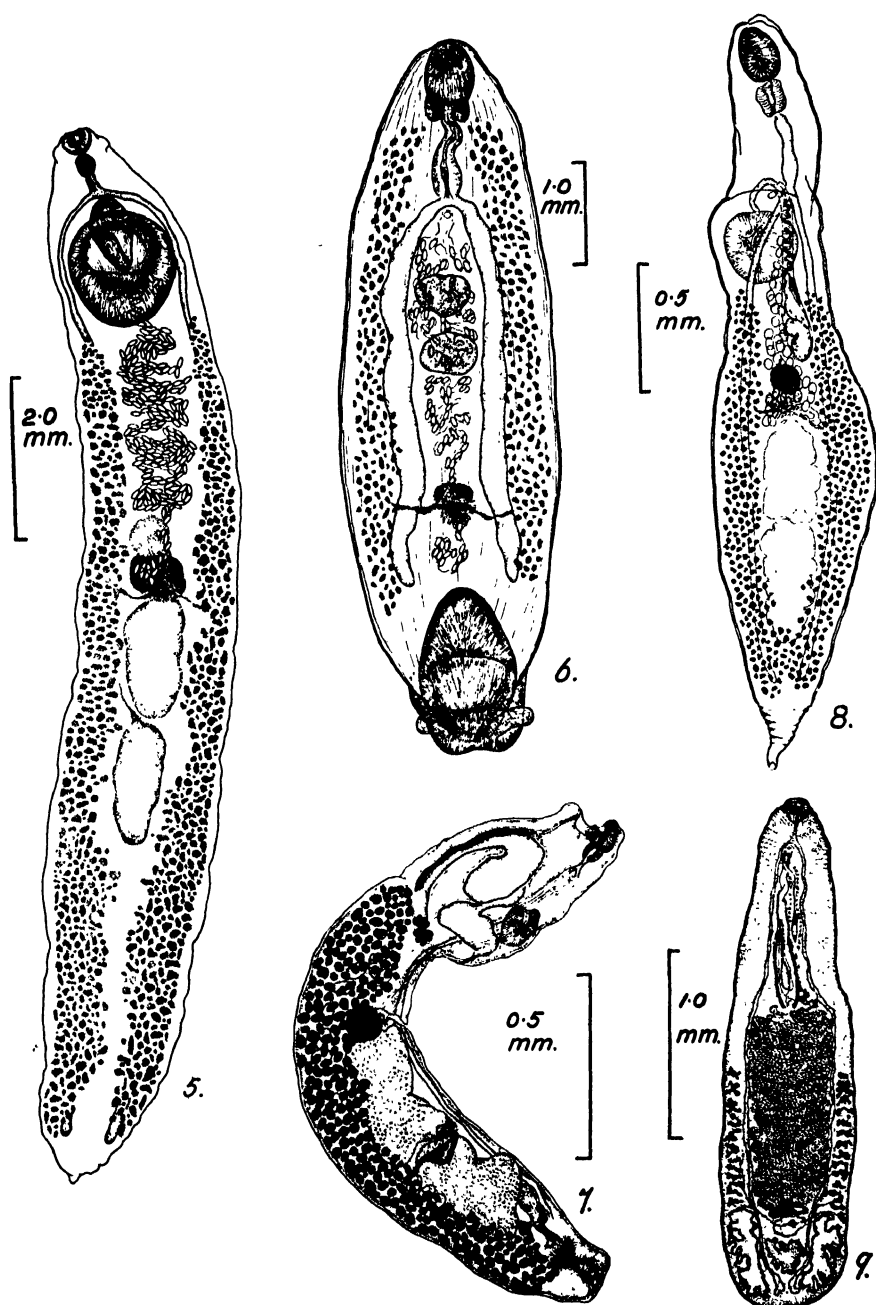
Ten specimens were found in the small intestines of four Black ducks, *Anas rubripes rubripes*, from the Lake St. Peter district, Que.

The fluke (Fig. 5) is elongate-oval in shape and quite flat, tapering slightly towards the ends, which are bluntly rounded. The average specimen measures 13.3 mm. in length by 2.1 mm. in width at the widest portion, in the testicular region. The head collars, in the specimens examined, are very rudimentary, and in the one illustrated measures 0.64 mm. in width. Most of the collar spines were lost, and those present did not afford the opportunity of making accurate measurements.

The sub-terminal oral sucker appears slightly drawn out posteriorly and is 0.4 mm. wide by 0.3 mm. in length. The oesophagus is very short, measuring 0.2 mm. in length. The intestinal caeca are narrow and tubular and reach to within 0.46 mm. of the posterior end of the worm. The pharynx measures 0.24 by 0.2 mm. The large acetabulum, 1.42 mm. in length by 1.3 mm. in width, is placed with its anterior margin 1.1 mm. from the anterior end of the worm. The acetabular opening is quite large, measuring 0.8 by 0.74 mm.

The testes lie one directly behind the other in the median line. The greater part of the anterior testis lies in the posterior portion of the anterior half of the worm, while the posterior testis lies wholly within the posterior half. The anterior testis measures 1.47 by 0.74 mm. and the posterior 1.51 by 0.62 mm. The testes are elongate-oval in shape and have entire margins. The cirrus pouch is large and elongate, extending slightly farther backward than the middle level of the acetabulum. The cirrus-sac contains a cirrus, a twisted *vesicula seminalis* and a well-developed *pars prostatica*. Both cirrus and metraterm open through a common genital pore of fairly wide aperture, directly posterior to the level of the caecal bifurcation.

The ovary, 0.5 by 0.48 mm., forms an almost perfect sphere with an entire margin. It lies a little to the left side of the median line with its posterior margin about 0.4 mm. anterior to the anterior margin of the anterior testis. A large and diffuse shell-gland occupies the greater part of the space between the ovary and testis. Ventral to the shell-gland there is a large vitelline reservoir. A uterine seminal receptacle lies close to the shell-gland and partly surrounded by it. The uterus lies in irregular coils extending from the ovary to the genital pore and bounded on either side by the intestinal caeca and vitellaria. The eggs are oval in shape and measure 82–120 by 60–70 $\mu$ . The



FIGS. 5-9. 5. *Hypoderaeum conoideum* (Bloch, 1782) non Railliet 1885, entire worm, ventral view. 6. *Zygocotyle lunata* (Diesing, 1836), entire worm, ventral view. 7. *Apotemon gracilis* (Rud., 1819) Szidat, 1929, entire worm, lateral view. 8. *Psilochasmus longicirratus* Skrjabin, 1913, entire worm, dorsal view. 9. *Notocotylus attenuatus* Rudolphi, 1809, entire worm, ventral view.

vitelline follicles occupy the lateral fields and extend from about the level of the posterior margin of the acetabulum to within 0.5 mm. from the posterior end of the body.

In several of the specimens examined there was a papilla-like extension of the cuticle at the posterior end of the worm.

Dietz (4) has reported *Ilypoderaeum conoideum* in the intestine of *Anas boschas*, and Yamaguti (18), from *Anas platyrhyncha platyrhyncha*. There appears to be no previous record of it from *Anas rubripes rubripes*.

*Zygocotyle lunata* (Diesing, 1836)

Several specimens of this trematode (Fig. 6) were taken from the caeca of the Black duck, *Anas rubripes rubripes*, and from the caeca of the domestic goose, *Anser domesticus*. The material was collected on the Island of Montreal. They do not differ from Stunkard's (13) and Travassos' (15) descriptions.

*Zygocotyle lunata* has been recorded many times from the caeca and intestines of many different species of wild and domestic ducks and geese in America. It has also been described as a parasite of the caecum and intestine of the ox. The writer has not been able to find any former record of the Black duck, *Anas rubripes rubripes*, being a carrier of this parasite.

*Apatemon gracilis* (Rud., 1819)

A considerable infestation by these Strigeids (Fig. 7) occurred in the small intestine of the American Golden-eyed duck, *Glaucionetta clangula americana*.

The specimens ranged from 1.38 to 2.72 mm. in length, the fore-body being 0.57–0.9 by 0.45–0.62 mm. and the hind-body 0.78–1.82 by 0.47–0.58 mm. The oral sucker, 0.14 mm. in diameter, is terminal and is followed directly by the smaller pharynx which is 0.1 by 0.08 mm. The acetabulum is somewhat larger than the oral sucker and measures 0.16 by 0.21 mm. The holdfast organ is composed of outer and inner lobes which join posteriorly at their bases. The outer lobe is considerably larger than the inner one. In the majority of specimens examined, both outer and inner lobes were retracted within the cup-like fore-body. At a position near the junction of the two lobes of the holdfast organ there is an adhesive gland which is apparently made up of a number of deeply-staining lobules.

The testes are large and lobed. The anterior one lies in the posterior part of the anterior half of the hind-body, while the other lies in the anterior part of the posterior half of the hind-body. The posterior testis, measuring 0.40–0.56 by 0.38–0.41 mm. was, in the majority of cases, larger than the anterior testis which measures 0.35–0.54 by 0.31–0.36 mm. The testes lie one behind the other and so close, in some cases, that they appear to be touching each other or even overlapping. The *vesicula seminalis* is quite a large structure and lies sigmoid-fashion, directly posterior to the posterior testis and close to the dorsal body-wall. The ejaculatory duct opens at the end of a bulbous genital cone.

Close to the anterior border of the anterior testis is the sub-globular ovary, measuring 0.18 by 0.17 mm. The vitellaria do not extend into the fore-body

but are confined to the ventral field of the hind-body; the follicles are separate and very numerous.

The writer has identified these specimens as *Apatemon gracilis* mainly because of the egg size and the disposition of the testes and ovary. The specimens adhere closely to the description given by Yamaguti (17) of the specimens he found in the small intestine of *Mergus merganser merganser*. This is the first time that *Glaucionetta clangula americana* has been reported as a host to *Apatemon gracilis*, although it has been previously recorded in the European Golden-eye.

*Psilochasmus longicirratus* Skrjabin, 1913

Worms of this species (Fig. 8) were recovered from the small intestine of the Black duck, *Anas rubripes rubripes* and the Canada goose, *Branta canadensis*. These are flat, fairly thick-set, and more or less lancet-shaped worms. The anterior portion, as far as the acetabulum, is conical in shape. The posterior portion is more flattened, becoming round again at the posterior end. From the posterior end of the worm a cylindrical, tail-like process is given off. This process is protrusible, being activated by numerous circular muscles which are plainly visible; it measures 0.29–0.75 mm. in length by 0.08–0.30 mm. at its widest point.

Several specimens having an average measurement of 3.05 mm. in length by 0.7 mm. in width were found, but a typically mature form is 5.73–6.30 mm. in length by 1.42 mm.–1.44 mm. in width at its greatest breadth in the anterior testicular region. The cuticle is unarmed and fairly smooth.

The sub-terminal oral sucker is 0.36–0.47 by 0.31–0.33 mm. in size. A pharynx, measuring 0.26–0.29 by 0.20–0.22 mm., is separated from the oral sucker by a very short prepharynx, and is followed by a fairly wide oesophagus, 0.55–0.63 mm. in length by 0.07–0.14 mm. in width. At a point about 0.16 mm. in front of the acetabulum the oesophagus bifurcates into the lateral intestinal caeca, which extend posteriorly to within 0.62–0.71 mm. of the posterior end of the worm. The acetabulum, in the anterior part of the second quarter of the worm, is borne on a thick stalk which protrudes 0.80–0.92 mm. from the ventral surface of the worm. It is 0.59–0.61 by 0.52–0.62 mm. in size.

The testes are post-equatorial and are situated one directly behind the other in the mid-line. Their margins are distinctly lobed. The anterior testis measures 0.82–0.85 mm. in length and 0.49–0.56 mm. in width, while the posterior is 0.67–1.00 by 0.46–0.52 mm. The cirrus-sac is very long and more or less club-shaped, with the large end of the club occupying the more posterior position. It is 1.56–1.65 mm. in length and 0.20–0.23 mm. at its widest posterior point. It encloses a seminal vesicle of fairly large size and a very long, convoluted cirrus. The posterior margin of the cirrus-sac extends to varying distances behind the anterior margin of the ovary. Both male and female organs open through a common genital pore which is situated at approximately the same level as the intestinal bifurcation and is somewhat sinistral in position.

The ovary is globular to sub-globular in shape and median in position. It measures 0.17–0.23 mm. in diameter. Directly posterior to the ovary is a diffuse shell-gland which separates the ovary from the anterior testis. Laurer's canal is present, with its pore to the left side of the median line. The uterus is short, with a few coils extending posterior and a few anterior to the ovary. The vitellaria occupy the lateral fields of the body and extend from shortly behind the ventral sucker almost to the posterior end of the body and inwards just to the intestinal caeca, with some follicles covering the caeca and a few extending inwards past them. Caudad of the posterior testis the two vitelline patches unite to form one. The vitelline follicles are comparatively small and densely packed. The eggs are relatively few in number and measure 82–97 by 70–76 $\mu$ .

The writer has been able to find only two other records of *P. longicirratu*s, both being from the Old World. Skrjabin (12) found specimens in the small intestine of *Fuligula nyroka* from Russian Turkestan, and Tubangui (16, pp. 384–386), found specimens in the domestic duck from the Philippine Islands. The author's specimens quite closely resembled those described by Skrjabin and Tubangui, but were slightly larger.

*Notocotylus attenuatus* (Rud., 1809)

A fairly heavy infestation by these trematodes occurred in the caeca and rectum of a Lesser Snow goose, *Chen hyperborea hyperborea*, from the eastern Arctic.

These trematodes (Fig. 9) are flat, narrowed anteriorly, and broadly rounded posteriorly, with the greatest width in the pre-ovarian region. The cuticle is densely covered with very small spines, the greatest number occurring on the antero-ventral surface. The average specimen measures 2.76 by 0.75 mm. On the ventral surface there are three rows of gland-like structures which open into protrusible pits. In the median row there are 14 such glands and in each sub-median row there are 15. As yet, the exact function of these glands is unknown.

The terminal oral sucker is cup-shaped and measures 0.13 by 0.15 mm. A pharynx is absent and the oesophagus is short, slender and approximately 0.15 mm. in length. The intestinal crura are long and slender, and terminate blindly about 0.07 mm. from the posterior end of the body.

The testes, 0.45 by 0.16 mm., are elongate, lobed, and situated one on either side of the ovary and separated from it by the terminal portions of the gut branches. The external seminal vesicle is tubular and lies in transverse coils at the base of the cirrus-sac. The cirrus-sac is long, club-shaped, and with the thicker end of the club placed proximal to the anterior coils of the uterus. The external surface of the cirrus-sac appears to be thrown into lancet-shaped evaginations which produces a pine-cone effect. The sac measures about 0.9 mm. in length and partially extends into the middle third of the body. The internal seminal vesicle is sacciform and occupies the base of the cirrus-sac. Continuous with the internal seminal vesicle is the *pars*

*prostatica*, which is more or less club-shaped and slightly convoluted. The common genital pore opens directly behind the caecal bifurcation.

The lobed ovary, 0.21 by 0.20 mm., lies median to the testes. Anterior to it and also in the median line lies an oval *ootype*, and directly anterior to this the diffuse shell-gland. The vitellaria are placed lateral to the intestinal caeca and extend from a point just posterior to the middle of the body to a point just anterior to the testis. The uterus lies in close transverse coils which extend from the base of the cirrus-sac to the region of the shell-gland, and which are bounded on either side by the intestinal caeca. The vagina is about half the length of the cirrus-sac and opens in the form of a slightly expanded funnel into the genital atrium. Surrounding the vagina are numerous irregularly shaped glands of unknown function. The uterine coils are distended by the countless numbers of oval eggs, which measure 24–27 by 10–12 $\mu$ . Each egg is provided with a single filament at either pole.

*N. attenuatus* is very cosmopolitan in distribution and not very particular as regards host specificity. It has been reported from many different species of ducks and geese, both domestic and wild. However, this appears to be the first time it has been recorded from *Chen hyperborea hyperborea*.

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## THE EFFECT OF DILUTION AND DENSITY ON THE FERTILIZING CAPACITY OF FOWL SPERM SUSPENSIONS<sup>1</sup>

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### Abstract

The effect of variations in dilution and numbers of fowl sperm injected into Brown Leghorn hens has been observed in a study of artificial insemination. Fertilization is influenced by the number of sperm introduced in a single injection into the vagina. The number of fertile eggs subsequently produced is affected when the number of sperm is about one hundred million or less, and none are fertile when the number falls below one million.

Asynthetic diluent containing sodium sulphate, glucose, and peptone supported motility *in vitro*, but affected the fertilizing capacity of fowl sperm in proportion to the percentage occurrence of the diluent in the suspension. Sperm serum, comprising about 75% of normal fowl semen, produces little harmful effect and appears to be a more favorable diluent. However, the development of the zygotes conceived by the sperm surviving an unfavorable medium is not impaired, and "hatchability" of the eggs remains unaffected.

By comparison of Walton's data for the rabbit with those reported here for the fowl, it is shown that rabbit sperm are more highly resistant to the "toxicity" of certain types of synthetic fluids. Also, it apparently requires 100 times more sperm in the fowl than in the rabbit for conception to result; this may be related to the comparative anatomy of the reproductive tracts.

### Introduction

As a result of investigations in which rabbit sperm secured from the *cauda epididymis* were diluted, in varying degree, with an isotonic sodium chloride solution and tested for fertility by artificial insemination, Walton (11) suggested that fertility rested on a quantitative basis. That is, the chances of conception following coition, as well as the size of the litter produced, depends not only on the number of ova shed but on the number of sperm available for their fertilization. Since the individual sperm become widely dispersed throughout the reproductive tract of the female, the chance of any one being in the vicinity of a fertilizable egg at the crucial time naturally depends on the total number injected. Consequently, there must be a point below which fertility commences to drop. Walton found that fertility was reduced when the number of sperm injected was less than one million, and complete sterility resulted when the number was less than ten thousand. He lists three factors that may have affected the results: "(i) The probability of any one spermatozoon reaching the fertilizable ovum is small; (ii) the spermatozoa are variable

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and not all capable of fertilization; (iii) toxicity may act differentially on sperm suspensions of varying density".

The first of these factors is the subject of this investigation. The attempt is made to prove that fertilization is less frequent when the number of sperm injected falls below a certain value, and to determine the approximate level at which no fertilization is obtained.

The second factor should not affect the results, since the proportion of non-functional sperm in the original sample remains the same, regardless of dilution.

As the sperm are suspended in a synthetic diluent that probably is not the most favorable environment, the third factor is most likely to have an effect on the results. If the functional efficiency of the sperm is affected by an unfavorable environment, it is only reasonable to suppose that an increasing proportion of the sperm would be rendered non-functional as the dilutions are increased. This might contribute to the decrease and ultimate cessation of fertilization as much as the decreased numbers of sperm. Since the amount of suspension injected in Walton's experiment was always the same, he was not able to measure separately the effect of dilution and of sperm number.

This question is of great importance, not only from the standpoint of practical artificial insemination, but also because of its bearing on the scientific concept of fertilization. Since Walton's results cannot be applied to the fowl, an experiment was designed to establish the degree to which fowl sperm can be diluted before fertility is affected. This must be known, approximately, before artificial insemination can be used intelligently as a tool for investigating the fundamental aspects of fertilization:

The fowl offers many advantages over the laboratory rodent as experimental material in the investigation of this problem. The hen remains consistently in oestrus or "in the lay" for long periods, and as fertilization normally follows mating at any time of day, one is not confronted with the necessity of synchronizing insemination and ovulation. This is rather difficult to accomplish in the rodent; and even in the rabbit, where ovulation can be induced by copulation with a vasectomized buck, it is often difficult to secure a number of does that will accept the buck at the same time. Furthermore, one copulation in the fowl serves to fertilize eggs for an average of about two weeks, and one can thus measure not only the production of fertile eggs within a given time limit, but also the length of time fertilization continues. In addition, the technique of artificial insemination in the fowl has been rendered very simple and efficient through the discovery by Burrows and Quinn (1) of the abdominal stimulation method of eliciting an ejaculatory reflex in the cock, and by the author's method (Munro (7)) of everting the vagina of the female to introduce sperm directly into the uterus. Finally, the male fowl possesses no accessory sex glands; the sperm are thus collected free from secondary secretions and have no tendency to coagulate as do the natural ejacula of rodents.

## Materials and Methods

The experiment, as originally planned, utilized six males and a group of about 60 females. The males were fully matured, about nine months old, at the beginning of the experiment. They had been regularly submitted to induced ejaculations during the preceding six weeks. The females were slightly younger and were just reaching the peak of their egg production. All birds were pure-bred Brown Leghorns, bred and reared at the Institute of Animal Genetics, Edinburgh.

All glassware used was thoroughly cleaned and neutralized. Owing to the small volume of the ejacula emitted by the cock (averaging about 0.5 cc. in the stock used) a rather delicate technique is required to effect the dilutions. After a few preliminary trials, it was found that they were most easily and accurately made by the drop method. A series of eight test tubes was used, and the semen was transferred from the small collecting dish to the first test tube by means of a finely graduated 1-ml. pipette with an attached rubber teat, the volume being measured and the number of drops counted in the process. The pipette was then thoroughly flushed with distilled water and with the diluent. Into each of the remaining seven test tubes were then delivered from two to four drops of the diluent; the actual number of drops depended on the volume of semen collected, but was the same in all tubes. The semen in the first tube was then thoroughly mixed by shaking, and a number of drops equal to that of the diluent was added with the same pipette to the first of the diluent-containing tubes. The thin layer of pure semen on the inner surface of the pipette was removed by flushing with distilled water followed by the diluent, and the contents of the test tube was then thoroughly mixed by sucking in and out of the pipette several times. This provided a 1 : 1 mixture of semen and diluent, or a 1/2 dilution. Likewise, by transferring from this tube an equal volume of the 1/2 dilution to the following tube, a 1/4 dilution was obtained. In this way an ascending series of dilutions—undiluted, 1/2, 1/4, 1/8, 1/16, 1/32, 1/64 and 1/128—each containing half as many sperm per unit volume as the preceding, was obtained.

In a series of preliminary trials it was shown, by counting with a Thomas-Hawksley haemocytometer, that the actual sperm density of each of these dilutions could be accurately predicted by estimations based on the observed density of the 1/2 dilution (Table I). Such estimations were found to be more accurate than those based on the sperm density of pure semen. In each trial, application of the  $\chi^2$  test for goodness of fit reveals that the differences between the observed and expected numbers fall within the range of chance. In the actual experiment, therefore, the sperm density of the 1/2 dilution only has been counted, the others being computed from this value.

The diluent used was that recommended by Milovanov (6) for rabbit sperm; it is relatively simple to prepare, supports motility *in vitro* longer than normal saline or Ringer's solution, and as long as the more complicated sodium and potassium phosphate solutions. Equal quantities of the eight dilutions were injected into hens that had laid on the same day, usually within four

or five hours. No eggs, therefore, were present in the uterus to interfere with insertion of the syringe and deposition of the semen. One bird was used for each dilution. The injection was made into the vagina by means of a finely graduated, all-glass, tuberculin syringe without a needle. The glassware was kept slightly warmed on a fibre-board square over an electric heater.

TABLE I

ACCURACY OF ESTIMATING SPERM DENSITY IN A SERIES OF DILUTIONS BY CALCULATION FROM THE OBSERVED DENSITY OF THE 1/2 DILUTION  
(Results of applying the  $\chi^2$  test are shown)

Trial No.		Pure	Dilution								$\chi^2$	P
			1/2	1/4	1/8	1/16	1/32	1/64	1/128	1/256		
I	Observed	384	179	84	37	18	10	5	3	2	2.992	0.89
	Expected	358		89.5	44.7	22.4	11.2	5.6	2.8	1.4		
II	Observed	363	195	126	43	22	11	6	3	—	11.223	0.08
	Expected	390		97.5	48.7	24.4	12.2	6.1	3.0	—		
III	Observed	349	174	85	41	23	13	—	—	—	0.664	0.96
	Expected	348		87	43.5	21.8	10.9	—	—	—		
IV	Observed	328	181	83	40	26	12	—	—	—	4.968	0.29
	Expected	362		90.5	45.2	22.6	11.3	—	—	—		

Following insemination, the hens were trapnetted and the eggs were marked and dated. These were placed in an incubator at weekly intervals. On the third day they were examined for fertility, and on the seventh and fourteenth days for dead embryos. By completing the records at hatching time, it was possible to determine if embryos hatched, or died during the first, second, or third weeks of incubation. Thus a record was kept for each egg laid by each hen after the artificial insemination. The protocol of a representative experiment is shown in Table II.

When a hen had produced consecutively five or more infertile eggs she was considered infertile until again inseminated, and was free to be used in a new experiment. A hen very rarely produces a fertile egg when the preceding five have been infertile. Therefore, this source of error can be safely ignored in the present experiment, especially since most of the hens were not used immediately after becoming infertile as indicated by this test.

Records of "hatchability" as well as fertility of the eggs were kept, to indicate the effect of pre-treatment of the sperm on vigor of the resulting embryo, apart from the ability to initiate conception.

All inseminations were performed between three and six p.m. Under these conditions only one egg was fertile out of 81 laid by fertile hens on the

TABLE II  
FERTILITY AND "HATCHABILITY" OF EGGS LAID AFTER ARTIFICIAL INSEMINATION

A typical protocol. The fertility and hatchability records are calculated on the data of the 2nd to 11th days, inclusive. I = infertile; H = hatched; D<sub>1</sub>, D<sub>2</sub>, D<sub>3</sub> = embryo died in 1st, 2nd, or 3rd week of incubation. Amount of fluid injected, 0.05 cc.

Dilution	Millions of sperm per cu. mm.	Hen No.	Days after insemination																			
			0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
Pure	9.54	960	I	I			H	H		I	H	I	I			I		I				
1/2	4.77	700	I		H		I	D <sub>1</sub>	D <sub>1</sub>		H		I						I			I
1/4	2.38	671	I	I			H	H	D <sub>2</sub>	D <sub>2</sub>	H		I				I					
1/8	1.19	776	I		H		H	H			H	H	I						I			
1/16	0.60	947	I	I			I	I	I		I	D <sub>2</sub>					I	I				I
1/32	0.30	686	I	I	I			I	H			I					I		I			
1/64	0.15	1001	I		I				I													
1/128	0.07	731	I	I					I													

first day following insemination. On the second day 88% of the eggs laid were fertile. This percentage did not increase on the third day. For purposes of comparison, fertility was calculated as the percentage of fertile eggs produced in a ten-day period beginning on the second day following insemination. "Hatchability" is the percentage of fertile eggs that hatched.

### Effects on Fertility

#### *(a) Dilution with a Synthetic Diluent*

Three experiments were conducted with each of six males. In the first, 0.05 cc. of the various suspensions was injected into each hen, in the second 0.2 cc., and in the third 0.3 cc. By thus varying the total amount of fluid injected and therefore the numbers of sperm, an additional means was provided for determining whether the differences were due to the degree of dilution or to the number of sperm injected. The results of the 18 experiments are shown in Table III. The results have been grouped into three divisions according to the volume injected, and within each division the results are listed by males, the latter being arranged in order of sperm density.

Occasionally a hen ceased to lay after insemination. Such instances account for the few cases where no data are given for a particular dilution. Pauses in egg production in individual birds are frequent, but these pauses are not more common following handling and insemination than at other times. Infertile hens that had laid just previous to insemination were scarce during the latter part of the experiment. When only seven of the desired eight were available, the test of the pure semen was omitted. This accounts for lack of data with the pure semen for five of the six males in the last division of Table III. However, this is immaterial since it is the degrees of fertility at the various dilutions in the three injection levels that are the important comparisons. The table shows that fertility drops rapidly when the semen is diluted. This begins even at the 1/2 dilution, which gives considerably poorer fertility than does pure semen; the decline is steady and reaches the zero point at a dilution of 1/64 in all levels excepting 0.30 cc., where one fertile egg is recorded out of 35 set at 1/128. Since this is the only fertile egg in all the data below 1/32, it can safely be regarded as a purely chance occurrence.

Two things in Table III very definitely indicate that it is not the decrease in total numbers of injected sperm that is responsible for the decrease in fertility with increasing dilution: (i) there is no increase in fertility as the amount of injected fluid increases, and (ii) within each of the three injection levels there is no tendency for fertility to be higher in the suspensions which are naturally denser. The sperm densities of the undiluted semen as estimated from the haemocytometer counts of the 1/2 dilution are shown in the table; since within each division the males are arranged in order of this estimated density, the fertility levels in the divisions should increase as the table descends. This does not occur even when there are five times more sperm in the semen of the last male than in that of the first, as in the second division. As there is little consistency in the density of the sperm samples from the individual

TABLE III  
EFFECT OF DILUTION AND SPERM DENSITY OF FOWL SEMEN ON FERTILITY AND HATCHABILITY OF EGGS LAID AFTER ARTIFICIAL INSEMINATION  
The numbers in each cell of the table indicate: top row—eggs set : fertile : hatched; bottom row—eggs fertile, % : eggs hatched, %.

Each hen injected with 0.05 cc. fluid									
Male No.	Millions of sperm per cu. mm.	Pure	1/2	1/4	1/8	1/16	1/32	1/64	1/128
52	2.84	7 : 7 : 7	6 : 4 : 2	5 : 4 : 4	8 : 3 : 3	5 : 0 : 0	4 : 0 : 0	—	—
		100 : 100	67 : 50	80 : 100	38 : 100	0 : —	0 : —	—	—
51	5.48	6 : 4 : 3	6 : 3 : 2	6 : 0 : 0	5 : 2 : 2	6 : 0 : 0	8 : 0 : 0	6 : 0 : 0	6 : 0 : 0
		67 : 75	50 : 67	0 : —	40 : 100	0 : —	0 : —	0 : —	0 : —
99	5.78	6 : 5 : 5	7 : 4 : 2	6 : 0 : 0	6 : 1 : 1	5 : 0 : 0	5 : 0 : 0	1 : 0 : 0	5 : 0 : 0
		83 : 100	57 : 50	0 : —	17 : 100	0 : —	0 : —	0 : —	0 : —
95	6.46	6 : 6 : 6	6 : 6 : 6	7 : 2 : 2	3 : 0 : 0	6 : 0 : 0	6 : 0 : 0	3 : 0 : 0	4 : 0 : 0
		100 : 100	100 : 100	29 : 100	0 : —	0 : —	0 : —	0 : —	0 : —
97	6.85	5 : 5 : 5	7 : 7 : 5	4 : 0 : 0	6 : 0 : 0	5 : 0 : 0	3 : 0 : 0	—	—
		100 : 100	100 : 71	0 : —	0 : —	0 : —	0 : —	—	—
50	9.54	8 : 5 : 5	8 : 6 : 3	6 : 6 : 5	7 : 6 : 6	8 : 3 : 2	5 : 1 : 1	4 : 0 : 0	4 : 0 : 0
		63 : 100	75 : 50	100 : 83	86 : 100	38 : 67	20 : 100	0 : —	0 : —
Unweighted mean		85.5 : 95.8	74.8 : 64.7	34.8 : 94.3	30.2 : 100	6.3 : 66.7	3.3 : 100	0 : —	0 : —
Weighted mean		84.2 : 96.9	75.0 : 66.7	35.3 : 91.7	34.3 : 100	8.6 : 66.7	3.5 : 100	0 : —	0 : —

TABLE III—*Continued*  
 EFFECT OF DILUTION AND SPERM DENSITY OF FOWL SEMEN ON FERTILITY AND HATCHABILITY OF EGGS LAID AFTER ARTIFICIAL INSEMINATION—*Cont.*  
 The numbers in each cell of the table indicate: top row—eggs set : fertile : hatched; bottom row—eggs fertile, % : eggs hatched, %.

Each hen injected with 0.20 cc. fluid									
Male No.	Millions of sperm per cu. mm.	Pure	1/2	1/4	1/8	1/16	1/32	1/64	1/128
50	1.94	7 : 7 : 7	7 : 3 : 1	7 : 2 : 2	6 : 2 : 2	4 : 1 : 1	—	2 : 0 : 0	6 : 0 : 0
		100 : 100	43 : 33	29 : 100	33 : 100	25 : 100	—	0 : —	0 : —
52	3.48	8 : 8 : 8	7 : 4 : 3	5 : 0 : 0	7 : 1 : 1	6 : 0 : 0	6 : 0 : 0	6 : 0 : 0	3 : 0 : 0
		100 : 100	57 : 75	0 : —	14 : 100	0 : —	0 : —	0 : —	0 : —
95	3.94	—	9 : 8 : 8	7 : 4 : 4	7 : 3 : 3	6 : 0 : 0	8 : 1 : 1	2 : 0 : 0	5 : 0 : 0
		—	89 : 100	57 : 100	43 : 100	0 : —	13 : 100	0 : —	0 : —
97	8.37	6 : 6 : 5	—	5 : 3 : 3	4 : 0 : 0	5 : 1 : 1	6 : 2 : 2	4 : 0 : 0	6 : 0 : 0
		100 : 83	—	60 : 100	0 : —	20 : 100	33 : 100	0 : —	0 : —
51	9.01	4 : 1 : 1	5 : 3 : 2	6 : 2 : 1	5 : 0 : 0	7 : 2 : 1	4 : 0 : 0	3 : 0 : 0	5 : 0 : 0
		25 : 100	60 : 67	33 : 50	0 : —	29 : 50	0 : —	0 : —	0 : —
99	9.84	8 : 6 : 6	8 : 0 : 0	6 : 4 : 4	7 : 1 : 0	7 : 0 : 0	7 : 0 : 0	7 : 0 : 0	8 : 0 : 0
		75 : 100	0 : —	67 : 100	14 : 0	0 : —	0 : —	0 : —	0 : —
Unweighted mean		80.0 : 96.6	49.8 : 68.75	41.0 : 90.0	17.3 : 75.0	12.3 : 83.3	9.2 : 100	0 : —	0 : —
Weighted mean		84.8 : 96.4	50.0 : 77.8	41.7 : 93.3	19.4 : 85.7	11.42 : 75.0	9.7 : 100	0 : —	0 : —

TABLE III—*Concluded*

EFFECT OF DILUTION AND SPERM DENSITY OF FOWL SEMEN ON FERTILITY AND HATCHABILITY OF EGGS LAID AFTER ARTIFICIAL INSEMINATION—*Cont.*  
 The numbers in each cell of the table indicate, top row—eggs set : fertile : hatched; bottom row—eggs fertile, % : eggs hatched, %.

Male No.	Millions of sperm per cu. mm.	Each hen injected with 0.30 cc. fluid									
		Pure	1/2	1/4	1/8	1/16	1/32	1/64	1/128		
52	497	5 : 5 : 4 100 : 80	8 : 7 : 5 88 : 71	5 : 0 : 0 0 : —	4 : 0 : 0 0 : —	6 : 0 : 0 0 : —	3 : 0 : 0 0 : —	5 : 0 : 0 0 : —	5 : 0 : 0 0 : —		
97	545	— —	6 : 5 : 4 83 : 80	6 : 1 : 1 17 : 100	5 : 0 : 0 0 : —	6 : 0 : 0 0 : —	6 : 0 : 0 0 : —	8 : 0 : 0 0 : —	6 : 0 : 0 0 : —		
95	673	— —	7 : 7 : 6 100 : 86	7 : 4 : 4 57 : 100	8 : 2 : 2 25 : 100	6 : 0 : 0 0 : —	8 : 2 : 2 25 : 100	6 : 0 : 0 0 : —	5 : 0 : 0 0 : —		
51	684	— —	7 : 6 : 3 86 : 50	8 : 0 : 0 0 : —	6 : 0 : 0 0 : —	6 : 0 : 0 0 : —	7 : 0 : 0 0 : —	6 : 0 : 0 0 : —	6 : 1 : 1 17 : 100		
99	820	— —	— —	7 : 0 : 0 0 : —	7 : 2 : 2 29 : 100	8 : 0 : 0 0 : —	6 : 0 : 0 0 : —	5 : 0 : 0 0 : —	5 : 0 : 0 0 : —		
50	922	— —	7 : 2 : 0 29 : 0	8 : 0 : 0 0 : —	8 : 2 : 0 25 : 0	8 : 0 : 0 0 : —	5 : 0 : 0 0 : —	7 : 0 : 0 0 : —	8 : 0 : 0 0 : —		
Unweighted mean			77.2 : 57.4	12.3 : 100	13.2 : 66.7	0 : —	4.2 : 100	0 : —	2.8 : 100		
Weighted mean			77.1 : 66.7	12.2 : 100	15.8 : 66.7	0 : —	5.7 : 100	0 : —	2.9 : 100		
General weighted mean		85.5 : 95.4	67.6 : 69.3	28.8 : 93.8	22.9 : 88.0	6.4 : 71.4	6.2 : 100	0 : —	1.1 : 100		

males, it might be thought that the technique of counting was at fault. However, the close agreement shown between the expected and the observed counts (Table I) refutes this suggestion.

A detailed discussion of the errors involved in estimating sperm density with the haemocytometer is given in a paper now in preparation. This subject, therefore, will not be discussed here. It may be mentioned that variations in the sperm density of semen samples from the same male occur naturally, and represent true differences rather than technical errors in counting. In fact, the sperm densities listed in the various tables of this paper are essentially correct within the limits of a small technical error and may be regarded as the actual densities. It would appear, then, from the data listed in Table III, that the diluent exerts a harmful effect that is proportional to the degree of dilution regardless of the density of the suspension.

This was critically tested by making three dilutions, 1/4, 1/16, 1/64, by diluting one drop of pure semen with the appropriate number of drops of diluent and injecting the total fluid. It was not possible to insure that the hen would retain the whole volume of higher dilutions after injection. As there was slightly over 3 cc. in the 1/64 dilution, the hen may have ejected a small amount of the fluid after her release, although all was retained at the time. Sperm samples from five different males were diluted in this manner and injected. Of each semen sample, one drop undiluted was also injected, making four levels for comparison. The results are given in Table IV. It is seen that

TABLE IV  
EFFECT OF INJECTING EQUAL NUMBERS OF SPERM WITH INCREASING AMOUNTS OF  
MILOVANOV'S DILUENT  
(Data presented as in Table III)

Male No.	Millions of sperm per cu. mm.	Pure	1/4	1/16	1/64
95	6.16	8 : 4 : 2	7 : 1 : 1	7 : 0 : 0	6 : 0 : 0
		50 : 50	14 : 100	0 : -	0 : -
50	7.18	6 : 3 : 1	6 : 6 : 6	5 : 1 : 1	7 : 0 : 0
		50 : 33	100 : 100	20 : 100	0 : -
52	7.80	6 : 6 : 4	5 : 1 : 0	8 : 1 : 0	4 : 0 : 0
		100 : 67	20 : 0	13 : 0	0 : -
51	8.66	8 : 1 : 1	7 : 1 : 1	2 : 0 : 0	8 : 1 : 1
		13 : 100	14 : 100	0 : -	13 : 100
97	10.20	8 : 8 : 6	8 : 1 : 0	8 : 0 : 0	8 : 1 : 1
		100 : 75	13 : 0	0 : -	13 : 100
Unweighted mean		62.6 : 65.0	32.2 : 60.0	6.6 : 50.0	5.2 : 100
Weighted mean		61.1 : 63.6	30.3 : 80.0	6.7 : 50.0	6.1 : 100

fertility falls rapidly with dilution of the sperm, and although the pure sperm gave lower fertility than is shown in Table III, the other levels are quite comparable. Obviously, the lowering of fertility as a result of dilution is due to differential toxicity, and the results of the first experiment afford no measure of the effect of the decreasing numbers of sperm injected.

Before the latter effect could be tested properly, a more satisfactory diluent was needed. Several other types of synthetic diluents were available for testing, including that especially recommended by Nikitina (8) for fowl sperm. However, as motility is generally recognized as a very good indication of fertilizing ability, and as the diluent used was found to maintain motility as long as any of several widely different types, it seemed unlikely that an ideal synthetic diluent could be obtained. Moreover, the task of testing biologically the efficiency of various diluents by their effects on fertility is rather a prolonged procedure. Therefore, an attempt was made to secure some natural fluid that would be more favorable and that might be used in an investigation of the immediate problem, *viz.*, the quantitative nature of fertilization. For this purpose sperm serum promised to be ideal.

(b) *Dilution with Sperm Serum*

The pooled semen from 14 or 15 males, comprising about 8 cc., yielded about 6 cc. of sperm-free serum after centrifuging for 15 to 20 min. at 2000 r.p.m. This serum is slightly milky although quite watery in consistency. A very occasional sperm cell may be found in the serum, but any such sperm are included in the estimated density of the dilutions made with serum, and

TABLE V  
EFFECT OF INJECTING EQUAL NUMBERS OF SPERM WITH INCREASING AMOUNTS OF SPERM  
SERUM AS DILUENT

(Data presented as in Table III)

Male No.	Millions of sperm per cu. mm.	Pure	1/4	1/16	1/64
51	4.38	6 : 5 : 3 83 : 60	6 : 6 : 6 100 : 100	5 : 2 : 2 40 : 100	
52	6.02	27 : 19 : 17 70 : 89	28 : 16 : 16 57 : 100	27 : 13 : 12 48 : 92	
97	7.72	11 : 9 : 7 82 : 78	— —	4 : 4 : 2 100 : 50	8 : 4 : 3 50 : 75
95	7.82	14 : 9 : 8 64 : 89	15 : 6 : 5 40 : 83	13 : 7 : 6 54 : 86	
Unweighted mean		74.8 : 79.0	65.7 : 94.3	60.5 : 82.0	50 : 75
Weighted mean		72.4 : 83.3	57.2 : 96.4	53.1 : 84.6*	50 : 75

thus do not introduce an error. In order to test the efficiency of the sperm serum a test was run, similar to that described above with synthetic diluent, *i.e.*, a series of three dilutions, 1/4, 1/16 and 1/64, containing the same number of sperm. Samples were secured from four of the males used in the previous test, diluted, and injected. When the volume of semen secured was sufficient, two preparations of each dilution were made and twice the number of hens injected. The results are given in Table V. It can be seen that there is but a slight drop in fertility as the dilutions increase. The results are compared graphically with the synthetic diluent in Fig. 1.

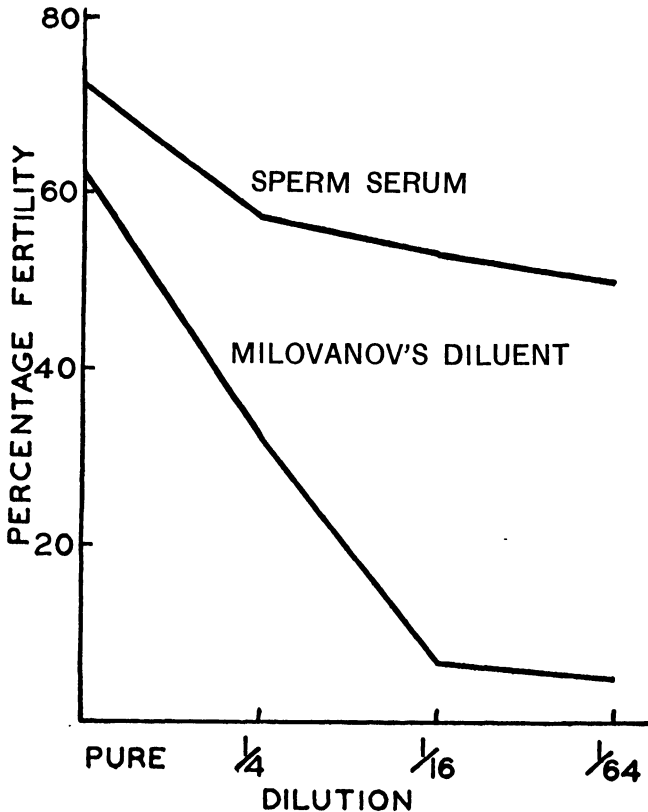


FIG. 1. Graph showing the superiority of sperm serum to a synthetic fluid when used as a diluent of semen, previous to artificial insemination. Same number of sperm injected at all dilutions.

Theoretically, equal levels of fertility are expected with the various dilutions. Several explanations may be given for the slight drop manifested. First, sera from different males probably differ slightly in chemical composition and may produce slight adverse effects on foreign sperm cells. Second, small amounts of the larger volumes may have been expelled by the hen. Third, experimental error may be responsible (*e.g.*, only one male was used in the 1/64 dilution). However, the fertility remained relatively constant when

compared to that secured under similar circumstances with the synthetic diluent. It appeared that the effect of decreasing sperm numbers could be measured by using sperm serum as a diluent, and an experiment to test this point was carried out.

By the time the investigation had progressed to this stage, the males had been maintained for nine months in individual cages. Although fed a complete ration they gradually lost appetite and weight, possibly owing to the development of excessively large combs, which interfered somewhat with eating and drinking through the grilled wire cage-fronts. The volume of the ejacula did not materially decrease, but motility tests indicated that the sperm were less vigorous than normally. Consequently, only two of the original males that had maintained their weight were used in this experiment. They were supplemented with three younger males that had been penned for some time on the floor and that were producing an average volume of microscopically normal sperm. The experiment was controlled by dividing the semen from each male into two parts; one was diluted to three levels,  $1/4$ ,  $1/16$ , and  $1/64$ , with varying volumes of serum but equal numbers of sperm in each; the other was diluted to the same three levels with equal volumes of serum, so that the total number of sperm in each was directly proportional to the dilution. In the latter, dilutions as low as  $1/256$  were also made and injected, but in the former it was not possible to inject more than the 3 cc. required in the  $1/64$  dilution. By comparing the results obtained with the two parts of the sample from each male, all variations in the vigor of different males and different ejacula from the same male can be eliminated, and the observed differences in fertilization ascribed to the numbers of sperm injected. Variations in fecundity were equalized by random selection of the hens. The results are given in Table VI and graphically compared in Fig. 2.

By application of the "Student's"  $t$  test to the means for each of the three dilutions in Table VI we get a  $t$  value of 4.914, the 0.05 point in Fisher's (2) table of  $t$  being 4.303. Thus, differences as great or greater than those observed between the two parts of the same sample, or between the different numbers of sperm injected, would be expected to occur by chance less than once in every 20 repetitions of the experiment.

In Table VI, the sperm density of the pure semen is given in millions per cu. mm., and in the next column this has been converted to the actual numbers injected in the pure semen. In the *a* rows, all dilutions contain the same number of sperm as the pure semen. In the *b* rows, the number of sperm injected decreases as the dilution increases; *i.e.*, in the  $1/64$  dilution the number of sperm is  $1/64$  of that given in Row *a* for the undiluted sample. These fractional numbers are not given in the table, except as a mean; they can, however, be readily calculated from the information given. In Fig. 2 they are marked at each of the plotted points.

It can be seen that fertility commences to drop at the first dilution ( $1/4$ ), although as many as 57,900,000 sperm have been injected. It continues to

TABLE VI

COMPARISON OF THE EFFECTS OF INJECTING A CONSTANT OR A DECREASING NUMBER OF SPERM  
DILUTED WITH SPERM SERUM

(Data presented as in Table III)

(a—equal numbers of sperm; b—decreasing numbers of sperm)

Male No.	Millions of sperm per cu. mm.	Millions of sperm injected	Pure	Dilutions				
				1/4	1/16	1/64	1/256	
98	3.87	193.5	13 : 4 : 3 31 : 75	7 : 4 : 4	7 : 1 : 1	5 : 1 : 0	—	
				57 : 100	14 : 100	20 : 0	—	
				7 : 1 : 0	8 : 0 : 0	6 : 0 : 0	3 : 0 : 0	
				14 : 0	0 : —	0 : —	0 : —	
1160	4.14	207.0	6 : 5 : 4 83 : 80	7 : 4 : 4	9 : 5 : 4	6 : 2 : 2	—	
				57 : 100	56 : 80	33 : 100	—	
				8 : 5 : 5	6 : 0 : 0	5 : 0 : 0	5 : 0 : 0	
				63 : 100	0 : —	0 : —	0 : —	
1663	4.22	211.0	12 : 9 : 9 75 : 100	5 : 4 : 2	7 : 4 : 4	7 : 5 : 5	—	
				80 : 50	57 : 100	71 : 100	—	
				7 : 1 : 1	7 : 4 : 2	6 : 1 : 1	—	
				14 : 100	57 : 50	17 : 100	—	
1695	4.84	242.0	3 : 1 : 1 33 : 100	7 : 2 : 2	6 : 1 : 1	5 : 1 : 1	—	
				29 : 100	17 : 100	20 : 100	—	
				4 : 3 : 3	7 : 1 : 1	6 : 0 : 0	—	
				75 : 100	14 : 100	0 : —	—	
97	6.08	304.0	15 : 12 : 10 80 : 83	7 : 5 : 5	8 : 6 : 5	8 : 8 : 7	—	
				71 : 60	75 : 83	100 : 88	—	
				6 : 3 : 2	8 : 3 : 3	6 : 2 : 1	6 : 0 : 0	
				50 : 67	38 : 100	33 : 50	0 : —	
Unweighted mean			a	58.8 : 82.0	43.8 : 92.6	48.8 : 77.6	—	
			b	60.4 : 87.6	43.2 : 73.4	21.8 : 83.3	13.2 : 75.0	0 : —
Weighted mean			a	57.6 : 89.5	45.9 : 88.2	54.8 : 88.2	—	
			b	63.3 : 87.1	40.6 : 84.6	22.2 : 75.0	13.8 : 66.7	0 : —
Mean number injected sperm (millions)			a	231.5	231.5	231.5	231.5	
			b	231.5	57.9	14.5	3.6	0.9

fall at a fairly steady rate until complete infertility is reached at a dilution of  $1/256$  or a total of 900,000 injected sperm. It should be noted that fertility obtained with the pure sperm in this and the experiments listed in Table V was about 20% less than that obtained in the tests done earlier in the year. It may be, therefore, that repetition of the last experiment at a season when fertility is higher might raise the levels of fertility somewhat in all dilutions, although this probably would not change their relative positions. As long as the comparisons are made within experiments, valid conclusions can be drawn concerning the effects of number of injected sperm and dilution.

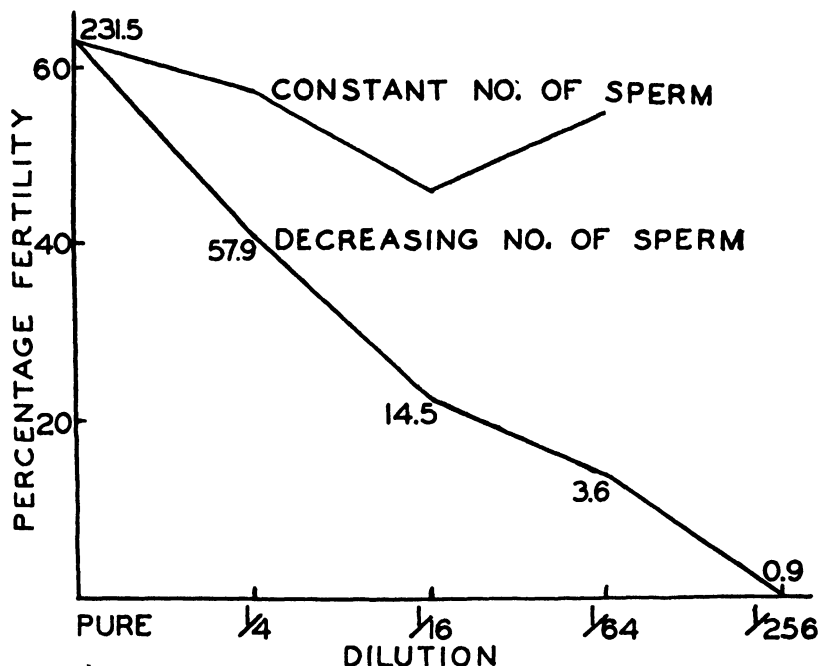


FIG. 2. A comparison of the effect on fertility of injecting a constant or a decreasing number of sperm at various dilutions. Sperm serum as diluent. Number of injected sperm in millions is shown on the curves.

In drawing general conclusions, therefore, one cannot be too dogmatic about the effects of sperm numbers on fertility. Quite possibly a small amount of fertility might be secured with as few as one million sperm in a more favorable season, and with larger numbers this might be correspondingly higher. However, it seems likely that any increase to be secured on repetition would manifest itself at the undiluted or slightly diluted levels. It is certain that enormous numbers of sperm, probably one hundred million or more, must be placed within the vagina of the hen before fertility will remain at a normally high level for a period of 10 days. When the number is reduced to fifty million, a noticeable drop in fertility occurs; when the number reaches one million, complete or nearly complete sterility ensues.

### Effects on Hatchability

Thus far, fertility has been considered and although the percentage hatchability has been listed in each table, only the former has been discussed. Hatchability, as used in this study, refers to the percentage of fertilized eggs that hatch, and is independent of fertility in computation. The recorded evidence concerning the relation between fertility and hatchability is rather contradictory. Under certain circumstances there appears to be a correlation between them (Pearl and Surface (10)), and Knox (4) found that the simple correlation coefficient measuring the degree of association between the two was  $+0.59$ . In practical poultry work it is the general experience of the breeder that the number of dead embryos is inordinately high during periods of low fertility. Possibly, this might be due to a lack of physiological vigor whereby a high proportion of non-functional sperm resulted in fewer fertilized eggs, and whereby a lack of normal vitality of the functional sperm caused a higher death rate in the resultant embryos. Such an explanation presupposes the existence within the sperm of varying protoplasmic states that affect embryonic development. This is a somewhat novel concept, not necessary to explain a correlation between fertility and hatchability; the correlation can be explained more satisfactorily by assuming variations within the egg that influence impregnation as well as embryonic changes. The large amount of complex food matter in the hen's egg provides a wide range for variation, making it more probable that the egg controls the developmental processes. The first possibility has been offered, however, since the data are pertinent to it. For instance, since the synthetic diluent decreased the fertilizing power of the sperm, probably by rendering a certain proportion non-functional, it is reasonable to suppose that a sub-lethal effect has been produced on at least some of those remaining functional. This supposition receives considerable weight when it is remembered that the proportion of non-functional sperm increased with the degree of dilution (Table IV), which may mean that there are varying levels of susceptibility between individual cells in the same suspension.

One might expect, therefore, that in suspensions in which large numbers of sperm have become non-functional, the functional sperm might be so affected that many of the embryos that they conceived would die before hatching. Inspection of Tables III and IV, however, serves to dispel any such notion. Table III shows that one fertile egg of 87 set from the  $1/128$  dilution, and six of 97 set at the  $1/32$  dilution, hatched. Statistical analysis reveals no correlation between fertility and hatchability at the various levels. Therefore, it appears that sperm that survive unfavorable treatment *in vitro* do not manifest their experience by any vital effect on the embryos they subsequently conceive. While we cannot generalize on this statement and conclude that the physiological state of the male parent is not reflected in the hatchability of the egg, we can advance the tentative hypothesis that once fertility is accomplished, the history of the developing embryo is largely controlled by the chemical composition of the egg. Such a theory excludes the effect of heritable genes

that may influence the development of the embryo. It applies only to seasonal or environmental variations in hatchability and has broader implications bearing directly on the general problem of embryonic mortality. It follows, for instance, that when fertility is high and hatchability low, the cause lies in the egg and is independent of the sperm; when fertility is low and hatchability normal, the male is at fault because either non-functional or insufficient numbers of sperm are produced; and when both are low, the egg at least is at fault and the sperm may possibly be.

### Discussion

One of the significant results of this study is the demonstration of the marked effect that the diluent has on the fertilizing capacities of the sperm. This suggests that similar effects must have influenced the results obtained by Walton (11) in the rabbit, and therefore, comparison of the data on the quantitative nature of fertility in the fowl (demonstrated with the sperm serum diluent) is not possible. However, a closer study of Walton's work shows that while the simple sodium chloride diluent that he used may have adversely affected the functional capacities of the rabbit sperm, the effect is far smaller than that of Milovanov's diluent on fowl sperm. The lowest dilution used by Walton was one part semen to ten parts diluent (*i.e.*, 1/10 in the terminology of this paper), and the series increased by multiples of 10 (*i.e.*, 1/10, 1/100, 1/1000, etc.). Although his results are tabulated in terms of sperm cells per unit volume, it appears that he secured fertility in some of the rather weak dilutions, at least as low as 1/10,000 and 1/100,000. At any rate, he secured no significant decrease in fertility until the total number of sperm injected was less than one million, and fertility ceased only when less than ten thousand were injected. When it is considered that these few sperm were suspended in as much as 3 cc. of fluid, there is little doubt that an enormously greater proportion of diluent was present in Walton's suspensions. As Milovanov's diluent is a more favorable fowl sperm diluent than normal saline, when judged by motility effects *in vitro*, it is clear that normal saline is either more satisfactory for rabbit sperm than for fowl sperm, or rabbit sperm is more highly resistant to unfavorable media of certain types.

Even admitting an adverse effect of the diluent in Walton's work—a possibility that he realized and referred to as "differential toxicity"—it is clear that the number required to ensure fertility in the rabbit is very much less than in the fowl. Optimum fertility in Walton's work was reached with about one million sperm, and in this work the indicated point is about one hundred million. Both minimum and maximum fertility in the rabbit seem to occur with only 1% of the numbers of sperm required to produce similar fertility levels in the fowl.

In this connection, however, it must be remembered that Walton injected a volume of 3 cc., whereas the present work is based on injections of 0.05 cc. While the volume may be relatively immaterial it seems possible that when the vagina of a rabbit is distended with a sperm suspension as in Walton's

work, some of the fluid might find a natural outlet by infiltration into the uterus and tubes. As the sperm would naturally be carried in their original concentration wherever the fluid penetrated in even the smallest amount, the transportation of the sperm would be aided. The exact mechanism of sperm transportation is still unknown; other factors in addition to the motility of the sperm are apparently involved (Hartmann (3), Parker (9)). It is generally agreed, however, that fewer sperm are encountered at successive levels of ascent within the Fallopian tubes of mammals, presumably because of the mechanism of transport. It can be seen that sperm introduced into the vagina or distal end of the uterus in concentrated form must find their way into the tubes by moving in the natural fluids. Thus they would be considerably handicapped compared with the same number suspended in a large bulk of fluid. Whether volume does assist fertilization depends on the penetration of the uterus and tubes by the injected sperm suspension, and therefore, on the level at which the natural mechanism begins to produce the usual thinning of sperm at successively higher levels. Presumably, volume might aid in the transportation of fowl sperm, as it appears to have done in the rabbit, but this has not been a factor in the present experiment, as the effect of sperm numbers on fertility (Table VI) was measured by injecting a volume of only 0.05 cc.

The fact that the quantitative basis of fertility in the fowl is on a much higher numerical level than in the rabbit is not surprising, in view of the comparative length, breadth and heavily ridged internal surface area of the oviduct of the fowl. Many more sperm would be required to seed the entire surface of this organ as thickly as the much smaller, although paired, mammalian analogue. This may explain the much higher density of sperm in fowl semen (about six million per cu. mm.) than in rabbit semen (one hundred thousand per cu. mm., Lloyd-Jones and Hays (5)).

### Acknowledgment

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# THE EGG-PRODUCING CAPACITY OF POPULATIONS OF *TRIBOLIUM CONFUSUM* DUV. AS AFFECTED BY INTENSIVE CANNIBALISTIC EGG-CONSUMPTION<sup>1</sup>

BY JOHN STANLEY<sup>2</sup>

## Abstract

Experiments are described in which adults of *Tribolium confusum* Duv. are maintained at 27° C., and 75% relative humidity in four different flour media: (a) ordinary whole wheat flour sifted through 76-mesh bolting cloth, (b) similar flour with from 30 to 135 *Tribolium* eggs per gm., (c) sifted whole wheat flour plus 3% of finely ground wheat germ and (d) flour plus germ plus eggs.

It is shown that when large numbers of eggs are eaten, there is a serious decline in egg production unless wheat germ in excess is also present. This is believed to be due to a scarcity of certain accessory growth substances found in wheat germ but not to the same extent in eggs. When ground wheat germ is present, the beetles seem to do somewhat better in the presence of eggs, possibly because of a better water supply, obtained from the eggs.

## Introduction

As a result of observations on some populations of *Tribolium confusum* in which the eggs were eaten as rapidly as laid, the writer suspected that the egg-laying rate of females in such populations was seriously reduced.

As the amount of flour consumed under these conditions of intensive egg eating is much less than normal, it seemed reasonable, in the light of the work of Sweetman and Palmer (2) on the accessory growth substances present in wheat germ and necessary to normal egg production, that this reduction in fecundity might be due to a lessened intake of these substances.

Accordingly, five experiments\* to test this hypothesis are described below. The results support the above supposition.

## Methods

All populations consisted of eight males and eight females, newly reared, and maintained in 32 gm. of medium at 27° C., and 75% relative humidity. Experiments Nos. 1, 2 and 3 were carried out in ordinary whole wheat flour which had been sifted four times through 76-mesh silk bolting cloth. In Expt. 1, the eggs were removed, counted and discarded every day, and the flour was changed every four days. The experiment was continued for approximately 33 days. The data are shown in Table I.

It is to be regretted that it was necessary to terminate Expt. 1 after 33 days, as this makes comparison with Expts. 2, 4 and 5, maintained for longer periods, somewhat less convincing. However, other data obtained in this

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\* It has been suggested that duplicate experiments should have been done for each medium. It should be noted that 16 insects are used in each group, so that each experiment has a greater statistical significance than if only one insect were used. There is in a sense an internal replication, and provided that all experiments are subjected to substantially identical environmental conditions, the labor and expense of replicating experiments of this type with *Tribolium* is hardly justified.

laboratory show that populations handled as in Expt. 1 do maintain their egg production relatively unimpaired for longer periods than 30 days. Several such tests provide the data of Table II.

In Expt. 2, an average of 30 eggs per gram was maintained in the flour for a period of approximately 35 days (Table III), by removing the flour and eggs every four days and replacing them by one-day-old eggs and fresh flour. Following this exposure to the eggs,

the beetles were placed in egg-free flour and the egg production per 24 hr. was determined for approximately 16 days.

TABLE II  
EGG-PRODUCTION RATES OF BEETLES LIVING IN  
SUBSTANTIALLY EGG-FREE FLOUR

Test	Time, days	Egg production, eggs per 24 hr.
A	0	52.23
	23.00	48.16
	74.03	47.61
B	0	70.08
	15.74	69.35
	30.97	69.00
	45.74	65.37
	60.17	61.80
	75.50	55.11
C	0	64.34
	50.33	63.25
	101.75	56.70

TABLE III  
DATA SHOWING COMPUTATION OF EGG-PRODUCTION  
RATES\* FOR EXPERIMENT No. 2

Time of test, days from start	Duration of test, days	Eggs produced	Rate, eggs per 24 hr.	Central time of test, days
34.96 to 38.94	3.98	68	17.08	36.95
38.94 to 43.07	4.13	91	22.03	41.00
43.07 to 47.75	4.68	82	17.52	45.41
47.75 to 50.83	3.08	66	21.43	49.29

\* Initial rate at  $T = 0$ , 68.65 per 24 hr.

TABLE I  
TOTAL NUMBERS OF EGGS PRODUCED IN EXPERIMENT  
No. 1

Time, days	Eggs	Time, days	Eggs	Time, days	Eggs
0.00	0	10.77	560	21.77	1128
.87	42	12.10	618	22.92	1190
1.86	90	12.79	664	23.75	1238
3.10	164	13.96	716	24.75	1290
4.03	218	14.78	762	25.79	1336
5.03	260	15.90	810	26.89	1390
6.05	316	16.77	862	27.87	1434
7.01	370	17.76	914	28.76	1476
7.75	404	18.78	976	29.84	1534
8.91	462	19.85	1032	30.78	1586
9.78	512	20.97	1078	31.74	1638
				33.85	1688

In Expt. 3, after an initial study had determined an average rate of 80.81 eggs per 24 hr., the beetles were placed for approximately eight-day intervals in flour with 50 eggs per gram, maintained as above. After each eight-day interval the egg production was determined for 24 hr. in egg-free flour, and the procedure was repeated. The data are shown in Table IV:

For Expts. 4 and 5, 3% of finely ground wheat germ was added to the flour and mixed for 24 hr. in a ball mill with a reduced load of pebbles. It was found necessary to use only a few pebbles; otherwise the mass was ground rather than mixed, becoming sticky and difficult to sift.

In Expt. 4, the egg production was determined for each four-day interval (Table V), the flour being changed

TABLE IV  
DATA SHOWING COMPUTATION OF EGG-PRODUCTION  
RATES\* FOR EXPERIMENT NO. 3

Time of test, days from start	Duration of test, days	Eggs produced	Rate, eggs per 24 hr.	Central time at test, days
9.98 to 10.97	.99	32	32.32	10.47
18.05 to 19.03	.98	29	29.59	18.54
25.92 to 27.92	2.00	63	31.50	26.92

\*Initial rate at  $T = 0, 80.81$  per 24 hr.

TABLE V  
TOTAL NUMBERS OF EGGS PRODUCED IN EXPERIMENT  
No. 4

Time, days	Eggs	Time, days	Eggs	Time, days	Eggs
0	0	69.16	3603	132.15	5836
3.90	239	73.07	3781	136.28	5912
7.95	492	77.22	3950	140.16	5959
12.00	747	80.94	4117	144.25	6013
16.10	994	84.91	4288	148.16	6076
20.11	1242	88.95	4448	152.30	6131
24.12	1475	93.12	4600	156.49	6185
26.87	1628	96.93	4779	160.20	6208
32.99	1954	101.06	4958	164.07	6243
37.19	2186	105.02	5122	168.14	6264
41.06	2382	109.22	5297	172.31	6293
45.17	2588	113.08	5326	176.09	6322
49.11	2765	117.19	5495	181.28	6354
53.09	2938	120.13	5588	184.16	6362
57.11	3100	124.18	5677	188.23	6381
60.93	3261	128.09	5760		
65.13	3450	129.30	5783		

TABLE VI  
DATA SHOWING COMPUTATION OF EGG-PRODUCTION  
RATES FOR EXPERIMENT NO. 5

Time of test, days from start	Duration of test, days	Eggs produced	Rate, eggs per 24 hr.	Central time of test, days
0 to 6.02	6.02	350	58.11	3.01
26.79 to 30.81	4.02	299	74.45	28.80
51.81 to 56.75	5.94	350	58.87	54.28
82.72 to 86.89	4.17	199	47.69	84.81
110.72 to 116.94	6.22	334	53.70	113.83
140.92 to 145.11	4.19	133	31.74	143.01
168.85 to 172.98	4.13	111	26.88	170.42
203.99 to 206.94	2.94	60	20.37	205.46

and the eggs discarded at each count. No extra eggs were added. The experiment was maintained for 188 days, when only six males and three females remained, and these were quite feeble. In Expt. 5 (Table VI), an average of 135 eggs per gram was maintained in the flour, except for a one-day test of egg production at approximately every 28 days. This concentration of eggs was obtained by adding all the eggs produced by a large stock culture of beetles. The number of eggs was estimated by weighing, and the mean computed at the end of the experiment. The number fluctuated from 80 to 190 per gram of flour. As eight males and eight females in 32 gm. of flour will eat eggs as rapidly as laid when the concentration is 30 eggs per gram, it is seen that the number used in this experiment would result in very intense egg eating. The writer is able to state, from other observations, that less than this number will result in the maximum possible egg consumption.

The resultant data were analyzed as follows: In Expts. 1 and 4, where repeated egg counts were available, cumulative egg curves showing the total production of eggs from the beginning of each experiment were plotted as in Figs. 1 and 3. Smooth curves were fitted to these by the Method of Least Squares, and the

resultant equations differentiated once to produce equations for the rate of egg production per 24 hr. From these equations the straight lines for Nos. 1 and 4, in Figs. 2, 4 and 5 were plotted.

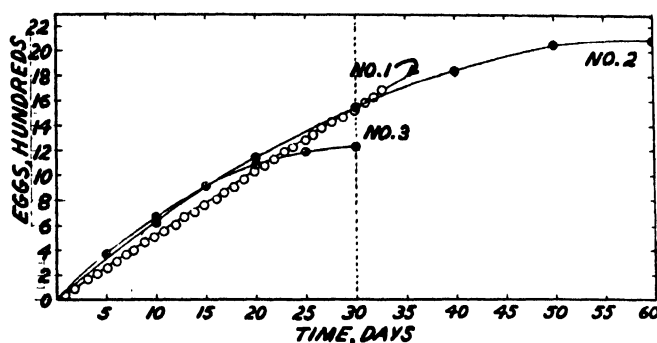


FIG. 1. Curves showing the total number of eggs produced from the beginning of Experiments Nos. 1, 2 and 3. Nos. 2 and 3 are plotted from equations obtained by integrating the equations fitted by the Method of Least Squares to the data of Nos. 2 and 3 of Fig. 2. No. 1 is a Least Squares fit to the points shown.

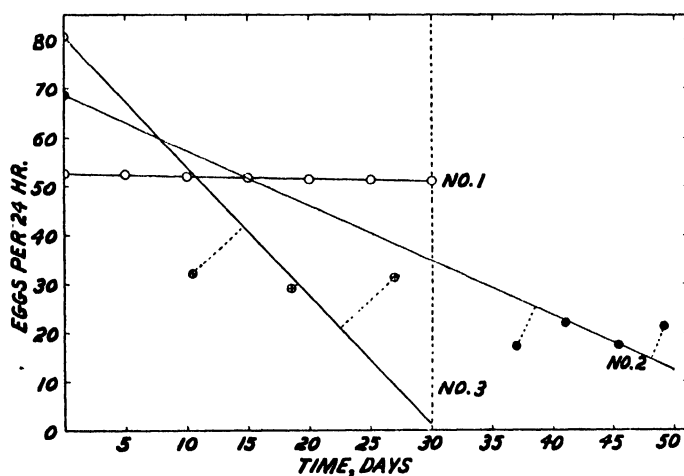


FIG. 2. Straight lines showing rates of egg production per 24 hr. for Experiments Nos. 1, 2 and 3. No. 1 is plotted from the first differential of the equation fitted by the Method of Least Squares to the data of No. 1 in Fig. 1. Nos. 2 and 3 are Least Squares fits to the points shown, with a modification of the computations which forced them to go through the values 80.81 and 68.65 at  $T = 0$  respectively, in order to show the decreases from these values.

In Nos. 2, 3 and 5 the data were in the form of rates of egg production observed at rather wide intervals. In these, a rate equation was fitted by the Method of Least Squares, and from the equations the straight lines Nos. 2, 3 and 5 of Figs. 2, 4 and 5 were plotted. These equations were then integrated once to obtain the equations of the smoothed cumulative egg production, and from the latter equations the smoothed curves Nos. 2, 3 and 5 of Figs. 1 and 3 were plotted.

These smoothed curves are shown only for comparison with Curves Nos. 1 and 4 of Figs. 1 and 3, and are not highly accurate representations of the course of events in Experiments 2, 3 and 5. They are based on the straight lines Nos. 2, 3 and 5 of Figs. 2 and 4, which are straight only by virtue of the assumption that they may be so fitted.

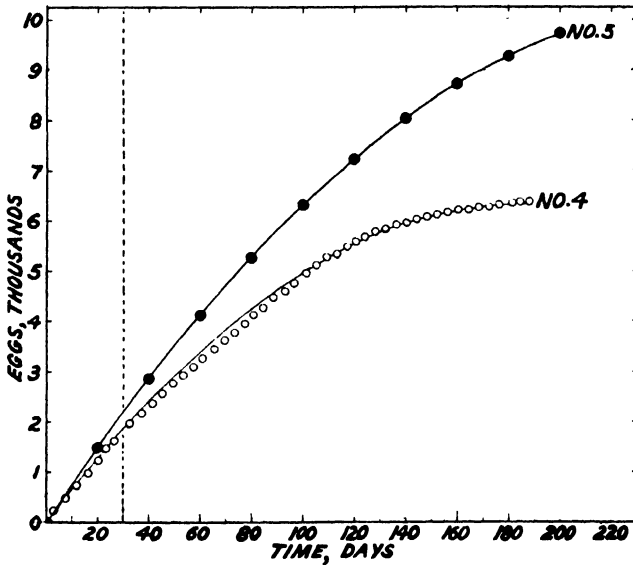


FIG. 3. Curves showing the total number of eggs produced from the beginning of Experiments Nos. 4 and 5. No. 4 is a Least Squares fit to the points shown. No. 5 is plotted from an equation obtained by integrating the equation fitted by the Method of Least Squares to the data of No. 5 in Fig. 4.

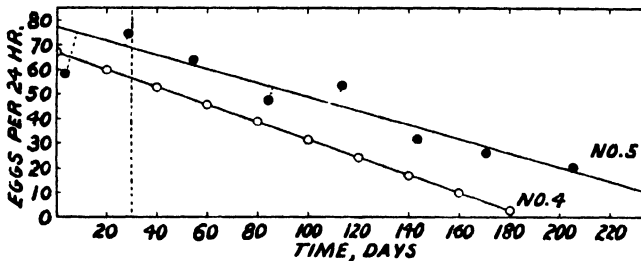


FIG. 4. Straight lines showing the rate of egg production per 24 hr. for Experiments Nos. 4 and 5. No. 4 is plotted from the first differential of the equation fitted by the Method of Least Squares to the data of No. 4 of Fig. 3. No. 5 is a Least Squares fit to the points shown.

It may be felt that the fit is not good in the straight lines Nos. 2, 3 and 5 of Figs. 2 and 4. In No. 5 of Fig. 4 this is due to experimental error. The experiments are not at all easy to carry out with accuracy, in spite of meticulous care with technique and excellent temperature control. In No. 2 it must be remembered that the effect of egg eating was no longer in operation when the egg production counts were made, so that production tended to remain substantially constant for the period studied. The discrepancies in

No. 3 may be due to the early death of the more susceptible females, leaving a hardy group whose egg-laying rate would probably have shown a steady downhill trend had the experiment been maintained for a longer period. In spite of this variation from a straight line, it was felt that, in comparison with Expts. 1 and 4, the same method of analysis should be applied throughout.

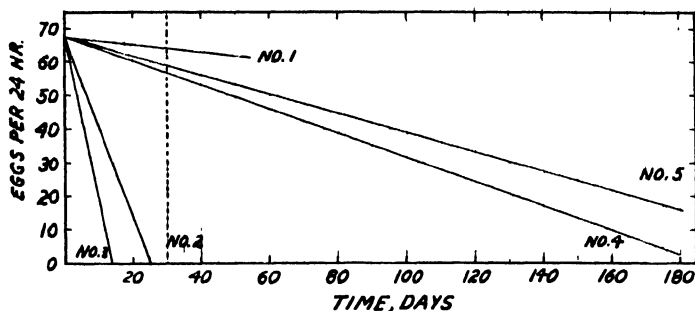


FIG. 5. Summary of the lines showing egg-production rates per 24 hr. for all five experiments, with the lines plotted as if all experiments had started with an initial rate of 67.49, the same as No. 4.

### Discussion

It will be seen from Fig. 1 that the cumulative egg curve (No. 1) for Expt. 1 (ordinary flour without added eggs) rose steadily and smoothly with a very small diminution in egg production (see Fig. 2), the reduction being from 52.56 per day at  $T$  (time) = 0 to 51.44 at  $T = 30$ . It is clear that the beetles were doing well in this medium, especially as none died during the 30 days.

In Expt. 2 (ordinary flour plus 30 eggs per gram) the initial rate was higher (Figs. 1 and 2), as this lot of beetles was selected to have a somewhat higher than average rate. This resulted (Fig. 1) in the population producing a few more eggs than in Expt. 1 in spite of the fact that in Expt. 2 there was a marked decline in egg production, from 68.65 at  $T = 0$  to 34.96 at  $T = 30$  and to 12.50 at  $T = 50$ . (The latter is a value computed on the assumption that the effect of egg eating had continued.)

In Expt. 3 (Figs. 1 and 2) the results are similar, the reduced egg production being even more marked, consistent with the more intense egg eating.

That the females were adversely affected can be seen from the fact that all lived to more than 30 days in Expt. 1, only four were left at 30 days in Expt. 2, one of which was sterile, while only three were still alive at 30 days in Expt. 3. These surviving females were dissected; those from Expt. 1 were found to be normal, while those from Expts. 2 and 3 had suffered a remarkable degeneration of their reproductive tracts, amounting in two beetles to almost complete destruction of the ovaries and oviducts. Some experiments have been performed to see if such damaged females will recover, but all have died in a few days or weeks, even when transferred to a medium consisting of as much as 50% of wheat germ.

When *Tribolium* beetles are young they are of a clear reddish brown color with a faint superficial translucency, but as they age they become darker,

almost black in some cases. It was noticed that the beetles of Expts. 2 and 3 showed this change very early, and also seemed to become sluggish, with all the appearance of illness.

The results of Expts. 4 and 5, in which 3% of ground wheat germ was added to the flour, are entirely different. In Expt. 4 (Figs. 3 and 4) in which no eggs were added, the egg production falls smoothly and steadily, but not excessively, dropping from 67.49 at  $T = 0$  to 56.74 at  $T = 30$ , and finally falling to 2.98 at  $T = 180$ , when only four males and one female were alive. These beetles were still quite active, but somewhat darkened. This is admittedly a more rapid decline in the early stages than occurred in Expt. 1, but the exceedingly small decline in egg production of the latter may have been due to the fact that as this was the first experiment, extraordinary care was used in removing the beetles from the flour. The flour was carefully turned over with a small brush, and the beetles dug out very gently. Time did not permit this method to be used in the other experiments, and the beetles were sifted out, although the writer had previously shown (1) that sifting was detrimental to egg production. However, a very gentle motion of the sieve was used, never violent enough to tear the beetles from their hold on the silk sifting-cloth.

In Expt. 5 in which a very large number of eggs was present, the decrease in egg production, so pronounced in Expts. 2 and 3, is almost absent (Figs. 3 and 4). The reduction was even less than in Expt. 4, in which no eggs were present. It is thought that this may have been due to a better supply of water, obtained from the eggs in Expt. 5. This supposition has no proof, but seems reasonable in view of the fact that *Tribolium* normally makes much of its water metabolically.

A summary of all five experiments is shown in Fig. 5, in which the straight lines for egg production per 24 hr. have been plotted with each experiment based on an initial egg-production rate of 67.49, the same as Expt. 4. This was chosen as the basic value because the insects of Expt. 4 were living in the standard medium now used for all experiments in the writer's laboratory. The drastic reduction in egg production caused by excessive egg consumption in the absence of a plentiful supply of wheat germ is clearly shown by the steep slopes of the lines for Nos. 2 and 3.

### Acknowledgments

The writer is indebted to the Science Research Committee of Queen's University for financial assistance in carrying out this and related work with populations of *Tribolium confusum*. He also wishes to express his appreciation of the careful work of Miss Isobel Hope and Miss Margaret Biehn, who looked after much of the routine work of the investigation.

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## THE REACTIONS OF THE HOUSEFLY, *MUSCA DOMESTICA* LINN., TO LIGHT OF DIFFERENT WAVE-LENGTHS<sup>1</sup>

By J. W. MACBAIN CAMERON<sup>2</sup>

### Abstract

Houseflies were reared on an artificial medium and tested with different wave-lengths of spectral light obtained from a quartz-mercury arc. The spectrum tested extended from  $\lambda 3022 \text{ \AA}$  to  $\lambda 5780 \text{ \AA}$ , and the lines were made of approximately equal intensity throughout. In addition,  $\lambda 5461 \text{ \AA}$  and  $\lambda 4078 \text{ \AA}$  were tested at several other intensities. The comparison standard in all cases was white light, obtained from a tungsten-filament, inside-frosted bulb, and filtered through copper sulphate solution. It was of constant quality, and the intensity was varied by changing the size of the bulb and by varying the distance from the bulb to the copper sulphate filter. The lighted areas to which the flies reacted were 5 by 10 mm. On one of these fell a total intensity of colored light of approximately 10.3 microwatts, on the other a range of intensity of white light of from 0.34 to 36.1  $\mu\text{w}$ .

Flies to be tested were removed from the breeding cage ten hours before tests began and were kept in darkness until used. Each fly whose record was used in compiling the final results was caused to make ten trips towards the two test lights, and a record was kept of the choice on each trip.

A description and discussion of the four different methods found in the literature for conducting experiments of this type, and for analyzing the results, are included. In the first method, the intensity of the test light of a given wave-length is kept constant, while that of the standard light, usually white, is varied until both are equally attractive.

The second method involves testing the colored light against a fixed intensity of white and finding the ratio of insects attracted to color. The intensity of white that will give the same ratio of attractiveness when tested against the standard is then determined.

In the third method, the two test lights are made equal in intensity, and their relative efficiency is considered to be directly proportional to the number of insects attracted to each.

In the last method, the standard is kept fixed in both quality and intensity, and the intensity of the test color is varied until the two are equal in attractiveness.

Application of the first three methods to the same data shows that they give results that vary greatly as the intensity changes. Some show that efficiency increases as the intensity increases, while others show a decrease in efficiency with increasing intensity.

If the intensities of all colored lights are equal, the three methods give practically the same qualitative results when applied to the same data. That is, a curve of efficiency is found which has its peak at the same wave-length, whatever method is used. Quantitatively, the results given by the three methods differ, so that no definite ratio of attractiveness can be determined between colors.

The data obtained were not amenable to analysis by the fourth method, but published results indicate that this is perhaps the best method for determining the quantitative relation between the stimulative efficiencies of light of different colors.

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The housefly, *M. domestica*, is much more strongly stimulated by ultra-violet light of wave-length 3656 Å than by any other part of the spectrum examined. The effect decreases, at first rapidly and then more slowly, as the longer wave-lengths are reached; it also decreases on the short-wave side of the peak. The spectrum available extended only as far as 3022 Å in the ultra-violet, at which point there was still an appreciable attractiveness, apparently greater than that of either yellow or green.

Several problems are suggested that require further investigation.

## Introduction

The importance of light in the existence of insect life has been known for many years but it is only within the last three decades that much progress has been made in the study of its effect. About 1890, the study of physiological effects of light was receiving considerable attention, but the apparatus and methods available at that time were soon exhausted, and for about twenty-five years the subject was almost forgotten. However, with the discovery of the effects of ultraviolet radiation in biology and the development of various sources of artificial light, together with the improvement of photometric methods, there has developed an increasing interest in the general problem of phototropic responses.

Many data are available on the response of insects to colors, but the larger part of the work appears to have been based on training methods. Following the method ordinarily employed, the test insects are trained to come to variously colored surfaces for food, and their ability to distinguish these colors is taken as a criterion of their ability to recognize different wavelengths of light. Qualitatively, such a method has much to recommend it, and very interesting results have been obtained; quantitatively, it leaves much to be desired. An important criticism of many experiments is that brightness has not been considered, or, if considered, has been inadequately corrected. Too frequently, spectral limits are almost disregarded and insufficient attention is given to the possibility of odor playing a part.

More recently the tendency has been to use spectral colors, either monochromatic or a narrow band, as the source of light, and to take the energy factor into consideration. Various methods of doing this have been followed, but it has been found that the results do not always agree (6, 88). This leads to the suspicion that some or all of the methods must be inaccurate, and shows the need for a more intensive study of methods, before quantitative conclusions can be drawn.

Apart from the purely scientific interest of such investigations there is the aspect of practical application. Larger and larger sums are being used every year in the fight to control insects. If some knowledge of the basic responses of insects to various factors in the environment can be accumulated, it is conceivable that more efficient means of control might be devised. With these thoughts in mind, the experiments to be described later were initiated.

Considerations of space have made it necessary to omit a survey of the literature, in which was included information taken from approximately 100 papers; only that which has a direct bearing on the present problem has been retained. The complete bibliography, however, is appended.

## Experimental

### TEST ANIMAL

The test animals used in these experiments were adult *Musca domestica* Linn. At first they were reared on horse manure, but this proved unsatisfactory, and eventually a synthetic larval medium was used, made as follows: wheat bran, 165 gm.; alfalfa meal, 88 gm.; dextrose, 4 gm.; dried yeast, 7 gm.; water, 600 cc. These were worked into a mash. This culture medium is a modification of that described by Richardson (85).

The above amounts were mixed for each dish of medium, and three dishes were kept in the breeding cage at all times. They were replaced in rotation at weekly intervals, and were covered with a wire cone after the first week. This cone had a small opening at the top so the flies could escape as they emerged, but it prevented the females from laying eggs in the older media. In this way the cage was kept well supplied with healthy flies.

For each day's tests, the flies were removed from the breeding cage in the morning, placed in individual homeopathic vials, 80 by 20 mm. in size, and stoppered with a plug of absorbent cotton dampened with two drops of distilled water. This amount of water was found most favorable; if the flies were kept all day with no moisture, mortality was very high, and if the plugs were too moist, the flies did not respond as well during the experiments. The vials were kept in a covered box in the darkroom until used.

Owing to difficulties with the electrical service, as well as in making measurements of intensity during the day, tests were usually carried out after 6.30 p.m. As a rule the flies were removed from the breeding cage about 8.30 a.m. On the few occasions when it was possible to test during the day, the flies were placed in their vials ten hours before the test began, as usual. Since the flies were kept in total darkness during this period, all the tests are strictly comparable.

Dolley (20) found that dark adaptation for one hour increased the sensitivity to light of the eye of *Eristalis tenax* by 21 times, this maximum being maintained for about two hours, after which sensitivity decreased rapidly to a point lower than that of the light-adapted eye.

Wolf and Zerrahn-Wolf (100) have made tests on dark adaptation in the honeybee, and they find an increase in sensitivity of about 1,000-fold in 25 to 30 min., after which the level remains constant. Unfortunately, as they apparently did not test the bees after more than 35 min. in darkness, it is not known whether there is a decrease similar to that reported for *E. tenax*.

If *M. domestica* behaves in the same way as these other insects, the prolonged period in darkness (originally due to circumstances rather than choice) is of distinct advantage in that the eye has reached its constant level of sensitivity. Possibly the results might be different with a shorter period of adaptation, i.e., the level of sensitivity might not vary in the same way for all wave-lengths. That, however, is a separate problem.

At first 15, and later 20, flies of each sex were used in each day's trials, and the results were analyzed separately. For the sake of uniformity, the

males were always tested first. In practically no tests did all the flies respond, as there were always some that died during the period of dark-adaptation, and some would give only a partial response (*i.e.*, would not make ten trips) or none at all. Results were not included in the total responses unless the flies made ten trips to the test lights.

### APPARATUS

Fig. 1 shows the apparatus used for testing the phototropic reactions, and Fig. 3 its plan. It was set up in a darkroom, and was essentially the same as that developed by Bertholf (6), with some alterations, which it is felt are definite improvements. The reaction box (*X*), supported on three adjustable legs, was made to the dimensions given by Bertholf, *i.e.*, 67 by 26 by 4 cm. inside, but instead of being of wooden construction with a screen top, it was made with the

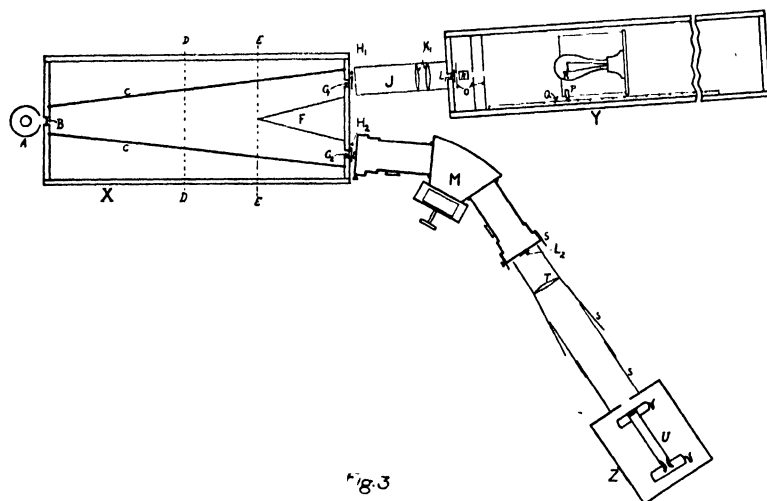


FIG. 3. Plan of apparatus for testing the reactions of flies to light. A, Pilot light. B, Ground glass screen. C, Glass sides of reaction box. D, Line from behind which flies started to light. E, Line which flies always passed to complete a response. F, Inner division of reaction box. G<sub>1</sub>, G<sub>2</sub>, Ground quartz screens. H<sub>1</sub>, H<sub>2</sub>, Synchronized shutters. J, Tube carrying lens and slit for white light. K<sub>1</sub>, Lens system for white light. L<sub>1</sub>, L<sub>2</sub>, Independent shutters. M, Monochromator. N, Copper sulphate filler. O, Diaphragms. P, Pointer. Q, Scale for setting white light. R, Source of white light—inside-frosted, tungsten-filament bulb. S, Tube to reduce stray light. T, Quartz lens. U, Quartz-mercury arc lamp. X, Reaction box. Y, Case for white light. Z, Case for arc lamp.

top and bottom of glass. Inside, partitions were placed so that the space in which the fly was liberated was in the shape of a broad Y. This was found necessary, as otherwise the flies would often get into the corners, where they were out of the field of light. The outer sides, C, of the Y were made of strips of glass painted on the inner side with flat black paint. These were spaced 6 cm. apart at the narrow end, and 22 cm. at the wide end. The inner V-shaped division, F, was a piece of galvanized iron covered with black crepe paper. It extended 19.5 cm. into the box, and the ends were spaced 10 cm. apart. The bottom of the box also was lined with the crepe paper. The paint and paper served to cut down reflection from the sides and bottom of the box,

PLATE I

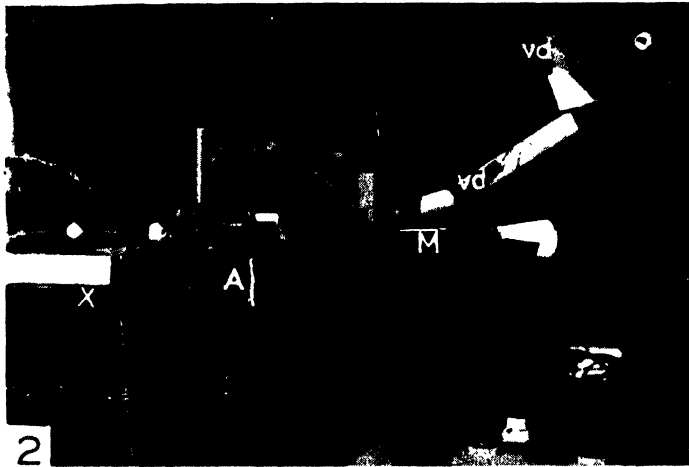
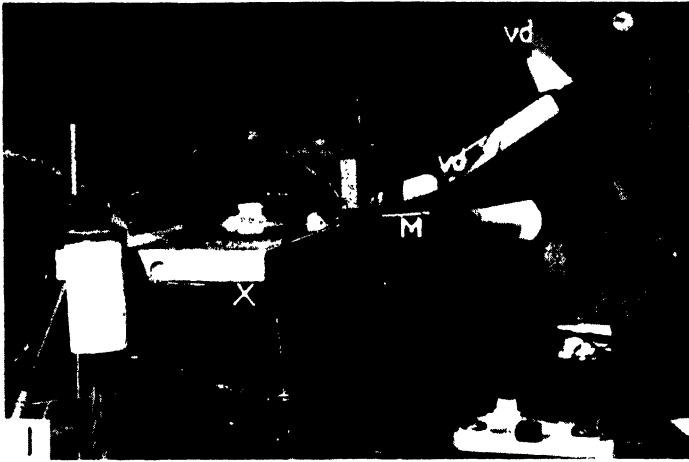


FIG. 1. Darkroom with apparatus for testing the reactions of flies to light. *M*, Monochromator; *vd*, Ventilating ducts; *X*, Reaction box in place.

FIG. 2. Darkroom with apparatus for measuring light intensity. *A*, Copper case containing thermopile, in position for measuring the intensity of white light; *M*, Monochromator. *vd*, Ventilating ducts; *X*, Reaction box moved to one side.



and, the top and bottom being glass, it was possible, by holding a light underneath, to find a fly which had strayed to a dark corner. For this purpose a ruby light was used, in order that the dark adaptation of the eyes of the fly might be disturbed as little as possible. The screens  $G_1$  and  $G_2$  were both of ground quartz and were centered in the ends of the arms of the Y, 16 cm. apart. The light fell on the ground side of these screens. Screen  $B$  was of ground glass and was made as a movable slide, so that the flies might be introduced and removed here instead of through a hole in the floor of the cage. Lamp  $A$  was a table lamp converted to a bull's-eye, instead of being in a box, thus allowing access to the opening at  $B$ .

The box  $Y$ , which contained the lamp producing white light, was about 142 cm. long by 19 cm. square. It had, on the exit end, a tube  $J$ , 20.3 by 6.3 cm., provided with a slit of such size that the light formed an image 5 by 10 mm. on the quartz screen  $G_1$ . This tube contained the lens system  $K_1$ , which was a combination of  $f:4.5$  approximately, so that the white light had an angle of divergence similar to that of the colored lights emitted from the monochromator  $M$ .  $L_1$  was a shutter used in cutting off the light from the thermopile while intensity measurements were being taken. The diaphragms  $O$  reduced the amount of stray light, and  $N$  was a glass cell 3 cm. in thickness filled with a 1.9% solution of copper sulphate.  $R$  was a Mazda 110-volt, inside-frosted, tungsten-filament bulb of 40-, 100- or 200-watt size depending on the required intensity of the white light. Its support was movable along a track, and it could be set by means of the pointer  $P$  and scale  $Q$  so that the filament was at any distance from 15 to 100 cm. from the filter  $N$ . This served as a guide for setting the lamp in position, and could not be used as a means of determining intensities, as the bulb  $R$  was not a point source, and the filter and lens interfered with intensity being determined by the law of inverse squares. The light intensity was measured before each series of tests.

The source  $U$  of colored light was a Hanovia Advanced Research Model 220-volt d-c. quartz mercury arc enclosed in a practically light-proof case  $Z$ . This lamp was used in conjunction with a Bausch and Lomb quartz monochromator  $M$ . During the course of the experiments two burners were used. The first one operated at 130 volts, consuming 3 amp. It developed a leak, and another arc was substituted which operated at 3.3 amp. and 125 volts. This substitution had no influence on results, as quality was determined by the setting of the monochromator prisms, and intensity by the width of the collimator slit of the instrument. Light was taken from the window at the positive end of the arc tube, and was focused by means of the quartz lens  $T$  on the collimator slit of the monochromator, from which the quartz dust cover was removed in order to reduce losses by reflection. Stray light in the room was reduced as much as possible by means of the tube  $S$ , which was made to telescope so that it could be separated and the arc and its housing could be tilted for lighting. In this system also an independent shutter  $L_2$  was placed so that the light beam could be interrupted when the intensity was

measured. A ventilating system, consisting of the ducts shown in Figs. 1 and 2 (*vd*) and a fan, carried the heated air from both light sources into the outer laboratory, thus keeping the temperature of the darkroom as low as possible.

The monochromator *M* was a Bausch and Lomb instrument, model 2800, in which all lenses and prisms were of crystal quartz. The drum for setting the instrument was graduated from 200 to 800 millimicrons. As the graduations were not quite accurate, all lenses and prisms were always set to give maximum intensity. Because the spectrum of the mercury arc has fairly widely separated lines, no error in quality would be introduced by this method. In order to eliminate possible change in quality, the series of tests with each wave-length of light was completed before starting the next, thus avoiding the necessity for resetting the instrument. Throughout the experiments the telescope slit was set at a width of 0.136 mm. (by microscopic measurement), as this gave an image 5.0 mm. wide on the screen *G*<sub>2</sub>. The collimator slit was set to give practically equal intensities of the different colors, except for green and violet, of which several intensities were examined. Owing to the internal reflection that commonly occurs in an instrument of this nature, the lights obtained were not truly monochromatic. For each setting the light was examined spectroscopically, and the results are listed in Table I.

TABLE I  
IMPURITIES OF SPECTRAL LIGHTS OBTAINED FROM MONOCHROMATOR, AS SHOWN BY  
SPECTROSCOPIC EXAMINATION

Wave-length in Ångstroms	Impurities
3022	Traces of 5780, 5461, 4970, 4359.
3132	Traces of 5780, 5461, 4359.
3656	Very faint 5780, 5461, 4359.
4078	Faint 5780, 5461; trace of 4359, 4108.
4359	Faint 5780, 5461; medium 4295, 4288; very faint background 4379 to 4288.
5461	Faint 5780.
5780	Faint 5890, 5857; trace 5461; faint background 5926 to 5857.
White	Continuous spectrum from 4130 to 6835.

Owing to the fact that the spectroscope was graduated at the short-wave end of the spectrum only as far as  $\lambda 4000 \text{ Å}$ , any ultraviolet impurities which may have been present are not included. An estimate, based on the apparent visual intensity of the different lines and therefore admittedly only approximate, is that in no case did the total impurity amount to 10% of the intensity of the spectral color desired.

The instrument for measuring light intensity is shown in position in Fig. 2 at *A*. It consisted of a Moll linear thermopile enclosed within a copper case, and mounted with a quartz lens of *f*:2.67 on a base that could be moved according to the wave-length being examined, so that the light was focused on the receivers of the thermopile. This was connected through a reversing

switch to a Zernike sensitive galvanometer, the scale of which was placed at a distance of about 2.5 m. from the mirror. Additional external resistances were also provided, to increase the range of intensities that could be measured.

In taking readings, the switch was closed and the zero noted, then the shutter (Fig. 3,  $L_1$  or  $L_2$ ) was opened and the deflection noted, the shutter closed and the zero again read. The two zeros were averaged, and subtracted from the deflection. The switch was then reversed, and the procedure repeated. The two values so obtained were averaged. Usually five such pairs of readings were taken, and the average value of all five, when corrected as indicated below, gave the intensity of the light for that particular series of tests. The thermopile-galvanometer combination was calibrated against a standard lamp obtained from the National Bureau of Standards, Washington, U.S.A., and a curve was drawn to show the relation between deflection and energy. From this curve the intensity of the different colors and of the different settings of the standard white could be read directly in absolute units.

The lens of the thermopile unit was arranged to collect all the light coming from the slit of the source, so that intensities are given as the total amount of light falling on the ground side of the quartz screens  $G_1$  and  $G_2$ . For monochromatic light this appeared to be the most satisfactory method available for determining the intensity. As applied to white light, it may be criticized because such light is by no means monochromatic, and hence a single lens would not focus all of it on the receivers of the thermopile. However, the light was always of the same quality (the intensity was varied by moving the bulb), and screen  $N$  acted as the source for the lens. Thus the lens was at a fixed distance from a source of constant quality, and consequently the proportion of the various wave-lengths falling on the receivers would be constant. As the lens was adjusted to give maximum deflection of the galvanometer, it is probable that the total error in measurement is quite small, and the results may be accepted as correct within, at least, the limits to be expected in the reactions of the flies themselves.

Considerable difficulty was experienced in making the actual measurements of light intensity. This was due to the fact that the intensity was very low, and so an extremely sensitive galvanometer had to be used. Static electricity affected this instrument to such an extent that it and its leads had to be thoroughly shielded, and practically all other articles in the room had to be grounded. Besides these precautions, it was necessary to keep the humidity in the darkroom above 60% in order that static charges on non-conductors might leak off as much as possible. As the galvanometer was not of the vacuum type, the wind also caused much disturbance, variations in air pressure caused by even a moderate breeze making the galvanometer so unsteady that accurate readings could not be taken. On these occasions tests with flies were not carried out, as it was found advisable to take intensity readings each night, in order to be sure that the instruments had not been moved out of alignment, or that no other source of error was present.

It was found that the operating condition of the lamps, and especially the voltage, must be kept within very narrow limits, as otherwise quite wide variations occurred in the intensity of the light produced. For this reason the lamp supplying white light was equipped with a rheostat and the voltage was controlled accurately at 110, a voltmeter being across the terminals of the lamp at all times. The arc lamp was provided with both a voltmeter and an ammeter and was operated at 125 volts, drawing 3.3 amp. This was regulated by a rheostat, and also by controlling the temperature by means of a damper in the ventilator duct. All three meters were checked and any necessary adjustments made before each fly was tested, and occasionally during a test if any fluctuations occurred on the power line.

Table II shows the intensities of the white and colored lights used, the values being the average of all readings taken for each intensity of white and for each wave-length. The intensities of the colored lights varied for the

TABLE II  
INTENSITIES OF LIGHT USED

White light		Colored light		
Size of bulb, watts	Intensity, $\mu\text{w.}$	Wave-length, $\text{\AA}$	Intensity, $\mu\text{w.}$ (males)	Intensity, $\mu\text{w.}$ (females)
200	36.10	3022	10.43	10.40
100	27.66	3132	10.09	10.09
100	19.82	3656	10.78	10.59
100	16.84	4078	9.77	9.71
100	11.39	4078	6.75	6.76
100	7.73	4078	4.41	4.42
100	6.45	4359	9.87	9.78
100	5.35	5461	21.90	21.47
100	3.30	5461	14.42	14.80
100	2.87	5461	7.94	7.94
100	1.46	5461	5.96	5.98
40	0.78	5461	4.10	4.12
40	0.34	5780	9.97	9.98
		White	10.78	10.78

two sexes on a number of occasions when the arc lamp became extinguished and had to be relighted and the intensity remeasured. On such occasions it was found that the light intensity was seldom exactly equal to that used before, and consequently the average of all results varies somewhat. The yellow light is indicated as being  $\lambda 5780 \text{ \AA}$ , because the monochromator would not separate the two yellow lines of the spectrum. The intensity values shown in Table II are believed to be correct within about  $\pm 10\%$ .

#### TECHNIQUE OF TESTING

The technique of testing the reactions of the flies was practically the same as that used by Bertholf in determining the effect of ultraviolet light on bees. When a fly was to be tested, the intensities of the white and colored lights

having been measured and recorded and the reaction box (Figs. 1 and 3, *X*) put in place, a vial containing a fly was taken from the covered box and the fly introduced through the opening by raising the glass slide *B*, screens *G*<sub>1</sub> and *G*<sub>2</sub> meanwhile being illuminated by the test lights. The fly was allowed to proceed until it had passed the imaginary line *EE*, and thus was definitely headed for one screen or the other. The shutters *H*<sub>1</sub> and *H*<sub>2</sub> were then dropped, and light *A* turned on, thus bringing the fly back to the starting point. The fly was always brought back at least as far as the imaginary line *DD* so that it was in the full field of light from both screens, and care was taken to see that it was on either the bottom or the top of the box, and not on one of the side pieces, *C*. This was a necessary precaution, as it was noticed that quite often a fly on the side would travel along it without showing any tendency toward a preference in choice of lights, sometimes in this way going repeatedly to the light known to be less attractive.

A possible explanation of this phenomenon is to be found in the results obtained by Hecht and Wolf (39) with the honeybee. They showed that the lower part of the compound eye is more sensitive to light than is the upper part. If a fly is on a vertical surface, as on the side piece of the reaction box in the present experiments, and is exposed to two lights that are side by side horizontally, then to the fly one is above the other, and more light from the source nearer to the vertical surface will reach the lower part of the eye.

TABLE III  
SAMPLE RECORD OF RESULTS

Date—11/III/38  
Wave-length—white  
Intensity—46.38 mm.\*\*

*M. domestica*—female  
White-distance—25(100)\*  
Intensity—15.47 mm.\*\*

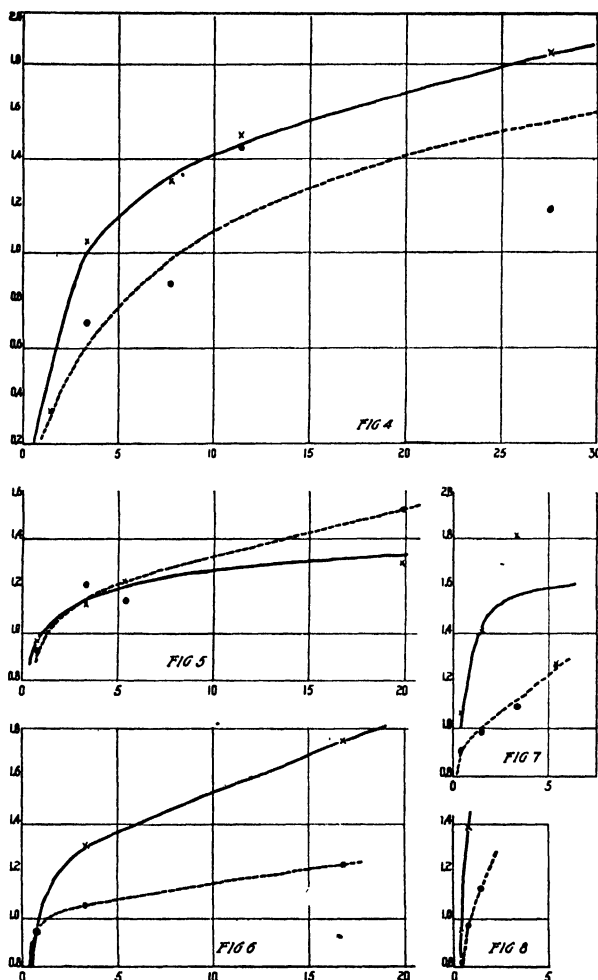
Fly No.	Response		Remarks
	Color	White	
1	1, 4-10	2, 3	Fair
2	1-4, 6-10	5	Fair
3	3, 5-10	1, 2, 4	Poor
4	1, 2, 4-10	3	Fairly good
5	3, 5-9	1, 2, 4, 10	Good
6	1-7, 10	8, 9	Good to fair
7	1, 2	--	Poor, no further response
9	—	--	No response
10	1, 2, 6, 7, 9, 10	3, 4, 5, 8	Poor
11	2-10	1	Fairly good
12	1, 2, 3, 5-10	4	Fairly good
13	1, 2, 4, 8, 9	3, 5, 6, 7, 10	Poor
14	2, 4, 8	1, 3, 5, 6, 7, 9, 10	Poor
15	1, 2, 3, 5-9	4, 10	Fair
16	1, 2, 4, 5, 8, 10	3, 6, 7, 9	Fairly good
17	1-7, 9, 10	8	Good
18	—	—	No response
19	1, 3, 4, 5, 7, 10	2, 6, 8, 9	Poor
20	1	—	Poor, no further response

\* 25(100) indicates that a 100-watt bulb was set at 25 in. on the scale. (Fig. 3, Q).

\*\* Deflection on galvanometer scale.

If this part, as in the bee, is more sensitive, then, even though the lights may be actually of equal stimulating value, the "lower" light will have a greater effect, and the fly will tend to go to it.

A record was kept of each trip, as shown in Table III. Sometimes it was necessary to stimulate the flies to respond by tapping the box with a light rubber mallet, and sometimes even this would not cause them to move. All such failures to respond were recorded. It is to this behavior that the words "good", "rather poor", etc., in the remarks column refer. "Poor" means



FIGS. 4-8. Abscissae, intensity of white light,  $\mu\text{w.}$  Ordinates, ratio attracted to white light. Males — x —. Females — o —. FIG. 4. Relation between attractiveness of green light  $\lambda 5461 \text{ \AA}$ , at an intensity of 21.90 (for males) and 21.47 (for females)  $\mu\text{w.}$ , and white light of varying intensity. FIG. 5. Relation between attractiveness of green light  $\lambda 5461 \text{ \AA}$ , at an intensity of 14.42 (for males) and 14.80 (for females)  $\mu\text{w.}$ , and white light of varying intensity. FIG. 6. Relation between attractiveness of green light  $\lambda 5461 \text{ \AA}$ , at an intensity of 7.94 (for males) and 7.94 (for females)  $\mu\text{w.}$ , and white light of varying intensity. FIG. 7. Relation between attractiveness of green light  $\lambda 5461 \text{ \AA}$ , at an intensity of 5.96 (for males) and 5.98 (for females)  $\mu\text{w.}$ , and white light of varying intensity. FIG. 8. Relation between attractiveness of green light  $\lambda 5461 \text{ \AA}$ , at an intensity of 4.10 (for males) and 4.12 (for females)  $\mu\text{w.}$ , and white light of varying intensity.

that not more than two responses were made without slight stimulation, "good" that at least eight trips were made unaided. The other classes were graded between these extremes. An interesting observation made during the course of the experiments was that such stimulation appeared to be more frequently necessary while testing the shorter wave-lengths, and especially the ultraviolet.

Each wave-length was tested against a number of intensities of white, so that a curve showing the ratio of response could be drawn. The intensities were chosen, as far as possible, to give a curve that would pass through the point of equal attractiveness. In one or two cases this could not be done, as will be explained. For each pair of lights, the test was repeated from two to six times, depending on the total number of flies reacting, and on the consistency of the results obtained in each test.

## Results

### EXPERIMENTAL DATA

The results were tabulated to show the ratio of flies going to the white light at each intensity and each wave-length. They are shown in Tables IV and IVa, and graphically in Figs. 4 to 20. The ratio of response to white rather than that to color is shown because, for each series of tests with a given wave-length, intensity of white was the variable factor while wave-length intensity was constant. Thus the curves show the effect on response to white light of varying its intensity, instead of the effect on response to color of varying the intensity of white. If the point sought for any series is that at which the two lights are equal in attractiveness, the ratio being 1.0, it is immaterial which way the data are plotted. For points other than equality, the ratio going to color is the reciprocal of that going to white.

The curve (Fig. 16) for  $\lambda 3656 \text{ \AA}$  is drawn with the abscissa at half scale, because it was so flat that otherwise it would have been impossible to extrapolate for the intensity giving a ratio 1.0. The lower intensities of white unfortunately were not tested against this wave-length.

At wave-length  $\lambda 5461 \text{ \AA}$ , no tests were conducted at the intensity used for the other wave-lengths. Therefore, for purposes of comparison, a value of  $10.32 \mu\text{w.}$  was chosen. This is the average of the intensities of all other colors used. The various values of relative attractiveness of this intensity required for the analysis of results were then interpolated from the appropriate graphs, *i.e.*, Figs. 12 and 13.

In all curves, the portion representing the attractiveness of low intensities of white is bent sharply downward. This was done on the assumption that if the variable white was extinguished, the flies would not go to that side of the reaction box. Consequently the origin was used as a point in plotting all curves. Examination of those graphs (*e.g.*, Figs. 17 to 19) in which the data are sufficient, shows that this assists in giving a well-fitted curve.

TABLE IV  
RESPONSES OF MALE *M. domestica* TO LIGHTS OF DIFFERENT WAVE-LENGTHS

Wave-length, Å	Intensity of color, $\mu\text{w.}$	Intensity of white, $\mu\text{w.}$	Number of observations	Ratio attracted by white	Wave-length, Å	Intensity of color, $\mu\text{w.}$	Intensity of white, $\mu\text{w.}$	Number of observations	Ratio attracted by white
3022	10.43	5.35	330	0.918	5461	21.90	1.46	130	0.340
		7.73	490	1.017			3.30	230	1.054
		16.84	360	1.169			7.73	60	1.308
		27.52	410	1.071			11.39	50	1.500
		36.10	140	1.692			27.52	110	1.852
3132	10.09	7.73	360	0.905	5461	14.42	0.78	240	0.967
		16.84	450	1.018			3.30	280	1.121
		27.52	490	1.095			5.35	200	1.222
3656	10.78	16.84	450	0.673			19.82	200	1.298
		27.52	400	0.812	5461	7.94	0.78	430	0.945
		36.10	380	0.910			3.30	120	1.308
4078	9.77	3.30	210	0.654			16.84	110	1.750
		5.35	260	0.844	5461	5.96	0.34	370	1.067
		7.73	320	1.013			1.46	320	1.406
		16.84	270	0.875			3.30	180	1.813
		27.52	290	1.015			5.35	250	1.273
4078	6.75	36.10	200	1.632	5461	4.10	0.34	380	0.959
		3.30	200	0.818			0.78	270	1.389
		5.35	300	1.000	5780	9.97	1.46	300	0.765
		7.73	310	0.950			2.87	390	1.053
		16.84	320	1.238			3.30	250	1.427
4078	4.41	27.52	270	1.523			5.35	320	2.077
		36.10	240	1.449			7.73	520	1.811
		6.45	240	0.727			16.84	290	2.333
		11.39	220	1.000			27.52	230	3.600
		27.52	240	1.425			36.10	240	2.559
4359	9.87	3.30	230	0.565	White	10.78	1.46	290	0.1935
		5.35	370	0.832			3.30	400	0.465
		7.73	340	1.048			5.35	400	0.613
		16.84	400	1.084			7.73	470	0.880
		27.52	260	1.031			16.84	380	1.360
		36.10	190	1.375			27.52	360	2.462
							36.10	460	2.332

### METHODS OF ANALYSIS

#### *Consideration of previous work*

Four methods for the determination of the relative stimulative efficiency of lights of different wave-length have been reported. In Method A, described by Mast (65), the energy of the test wave-length is kept constant, while that of the standard (white) is varied until the lights are equally attractive. This is the method used by Bertholf (6) in studying the reactions of bees to ultra-violet light.

Method B is a modification of A, made by Bertholf (5) in his study of the reactions of bees to different regions of the visible spectrum. This method involves testing each wave-length against a fixed intensity of white and

TABLE IVa  
RESPONSES OF FEMALE *M. domestica* TO LIGHTS OF DIFFERENT WAVE-LENGTHS

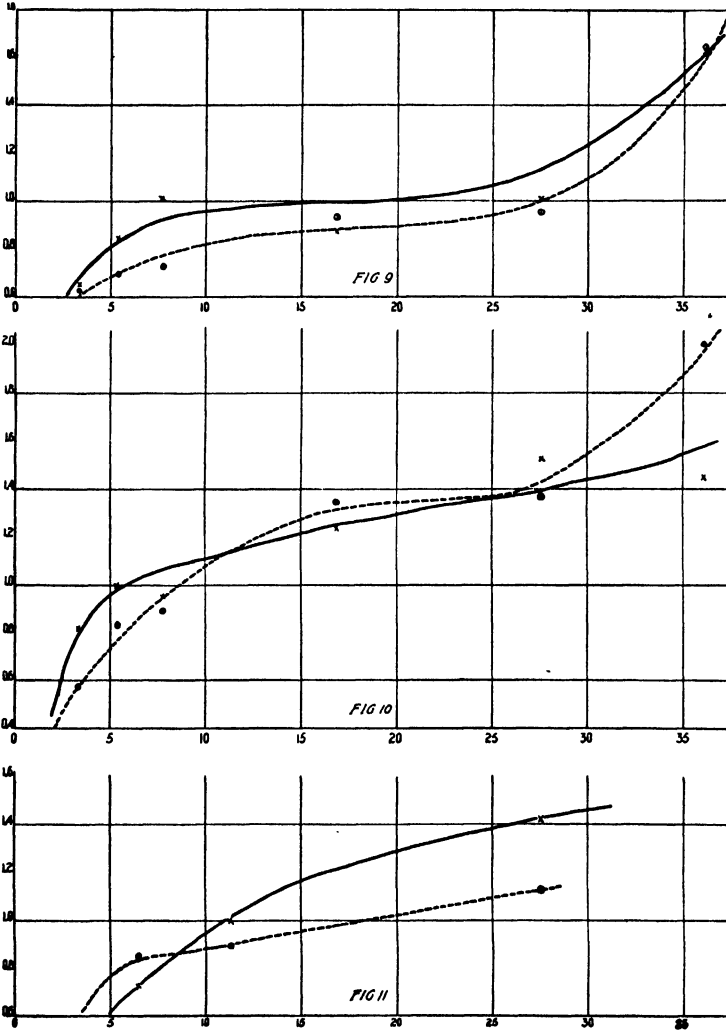
Wave-length, Å	Intensity of color, $\mu$ w.	Intensity of white, $\mu$ w.	Number of observations	Ratio attracted by white	Wave-length, Å	Intensity of color, $\mu$ w.	Intensity of white, $\mu$ w.	Number of observations	Ratio attracted by white
3022	10.40	5.35	440	0.630	5461	21.47	3.30	250	0.701
		7.73	530	0.636			7.73	60	0.875
		16.84	530	1.104			11.39	120	1.450
		27.52	560	1.171			27.52	70	1.188
		36.10	210	1.307					
3132	10.09	7.73	350	0.591	5461	14.80	0.78	250	0.923
		16.84	360	0.846			3.30	150	1.206
		27.52	420	1.049			5.35	190	0.959
3656	10.59				5461	7.94			
		16.84	440	0.636			0.78	440	0.947
		27.52	420	0.867			3.30	210	1.059
4078	9.71	36.10	380	0.900	5461	5.98	16.84	120	1.222
		3.30	200	0.626			0.34	420	0.901
		5.35	380	0.697			1.46	230	0.983
		7.73	390	0.726			3.30	140	1.090
		16.84	430	0.937			5.35	260	1.261
4078	6.76	27.52	470	0.958	5461	4.12			
		36.10	220	1.650			0.34	80	0.818
							0.78	370	0.978
							1.46	230	1.130
4078	4.41	3.30	310	0.574	5780	9.98	1.46	330	0.737
		5.35	350	0.833			2.87	410	1.370
		7.73	410	0.889			3.30	240	1.400
		16.84	490	1.345			5.35	420	1.782
		27.52	410	1.370			7.73	560	1.732
4078	4.41	36.10	310	2.010	White	10.78	16.84	420	2.652
							27.52	280	3.828
							36.10	220	4.947
4359	9.78	6.45	230	0.855	White	10.78			
		11.39	250	0.894			1.46	330	0.218
		27.52	240	1.124			3.30	430	0.458
							5.35	410	0.444
							7.73	390	0.797
4359	9.78	16.84	390	1.080	White	10.78	16.84	420	1.080
		27.52	300	1.098			27.52	410	1.662
		36.10	230	1.212			36.10	450	2.082

determining the ratio of attractiveness. Then a white of variable intensity is substituted, and the intensity which, when tested against the standard, has the same ratio of attractiveness as that of the wave-length, is determined. In both methods the value found is corrected to allow for differences in intensity of the color. The result is taken as the "relative stimulative efficiency" of the color tested.

Method C, used by Sander (88), involves making the standard and the test wave-length equal in intensity, and determining the numbers of test animals attracted to each, this being taken as a measure of the relative efficiency. In Method D, also used by Sander, the test wave-lengths are adjusted in intensity until they are equal in attractiveness to a standard that is fixed in both intensity and quality. The relative efficiencies of the tested lights

are considered to be proportional to the intensities so found, because these intensities are found to be equally attractive when tested together.

In a discussion of his method, Mast states that by following the procedure recommended, the stimulative effect of the different wave-lengths tested is



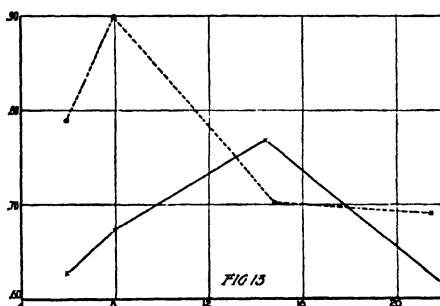
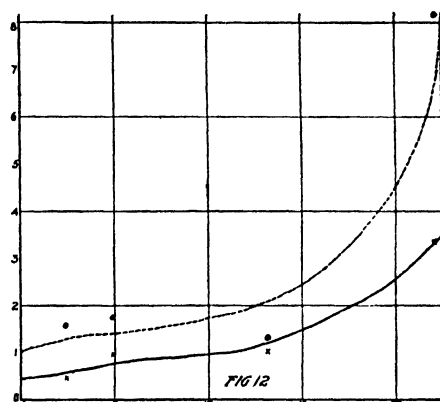
FIGS. 9-11. Abscissas, intensity of white light,  $\mu\text{w.}$  Ordinates, ratio attracted to white light. Males— $\times$ —, Females— $\circ$ —, FIG. 9. Relation between attractiveness of violet light  $\lambda 4078 \text{ \AA}$ , at an intensity of 9.77 (for males) and 9.71 (for females)  $\mu\text{w.}$ , and white light of varying intensity. FIG. 10. Relation between attractiveness of violet light  $\lambda 4078 \text{ \AA}$ , at an intensity of 6.75 (for males) and 6.76 (for females)  $\mu\text{w.}$ , and white light of varying intensity. FIG. 11. Relation between attractiveness of violet light  $\lambda 4078 \text{ \AA}$ , at an intensity of 4.41 (for males) and 4.42 (for females)  $\mu\text{w.}$ , and white light of varying intensity.

directly proportional to the various illuminations from the white light required to make the organisms in each of the different conditions proceed in the same direction. It is shown later that this reasoning is only qualitatively correct.

Bertholf (3-6) has conducted extensive investigations on the reactions of the honeybee to light, using a modification of Mast's apparatus. He placed the two test lights side by side, and found the intensities required to make 50% of the test animals go to each in a large number of trials. The principle of the method used in testing the reactions to ultraviolet light was essentially the same as Mast's, but was modified somewhat in testing the visible part of the spectrum. Combining the results from these two investigations, Bertholf (6, p. 711) obtained a curve representing the response of the honeybee to the different spectral regions from wave-length 280  $m\mu$  to 700  $m\mu$ . This curve shows two apparent maxima of stimulation, one in the so-called visible part of the spectrum at 553  $m\mu$ , and one about four and one-half times as great in the ultraviolet at 365  $m\mu$ . In the visible spectrum, it appears that the bee is considerably less sensitive to yellow, orange and red than is the human species, but is more strongly stimulated by blue and violet.

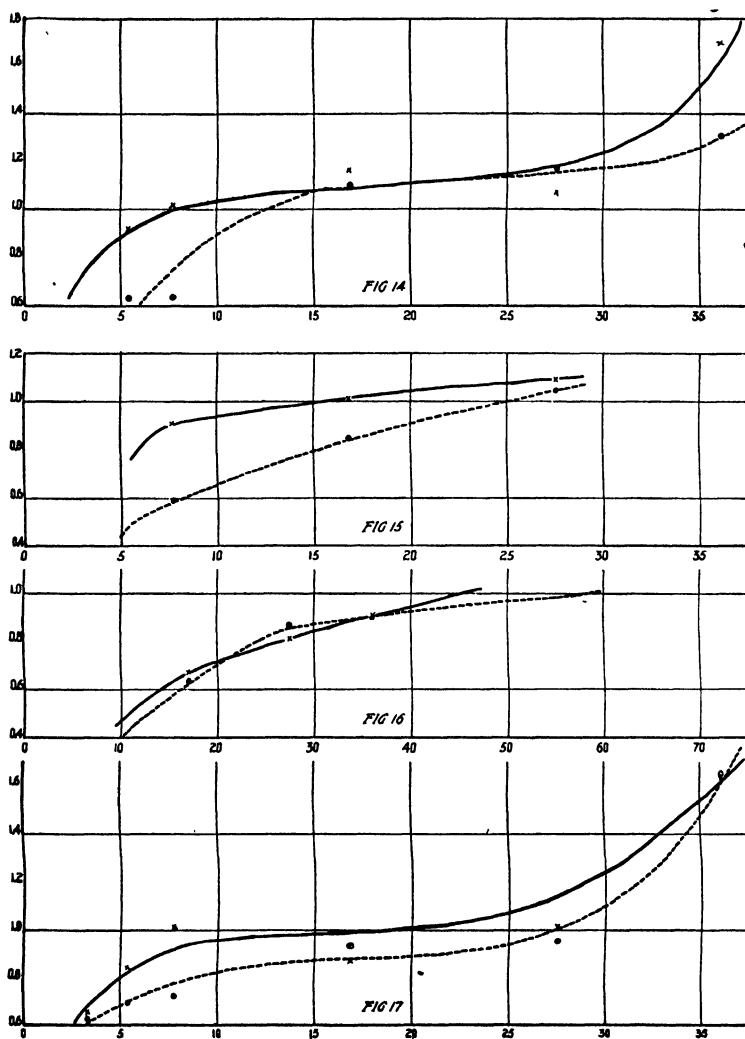
Sander (88) also tested the response of the honeybee to light, but his conclusions were very different from those of Bertholf. His method differed in that he first made all his lights of equal intensity, and then tested each wave-length against all others used. He found two maxima of stimulation but they were at about 570 and 470  $m\mu$ , with a minimum at about 520  $m\mu$ , and with a steady decrease from 470  $m\mu$  into the ultraviolet. Sander explains the discrepancy between his results and those of Bertholf on two grounds. Firstly, Bertholf did his work in two parts, beginning with tests of the reactions to visible light (5), and later examining the ultraviolet region (6).

The results were then brought to the same numerical range by calculation, and this, in view of the fact that the second set of results had to be multiplied by 5206.7, might introduce a very large error. Secondly, Sander points out that Bertholf's experiments were conducted with lights that varied in energy content, and again the results were adjusted by calculation. That is, the most intense wave-length (365  $m\mu$ ) was given a value of 100, and the others were rated accordingly. These values of intensity were then divided into



FIGS. 12-13. Males —X—. Females ---O---.  
FIG. 12. Variation in attractiveness of green light  $\lambda 5461 \text{ Å}$  with varying intensity (Method A). Abscissa, intensity of green,  $\mu w$ . Ordinate, intensity of equally attractive white,  $\mu w$ . FIG. 13. Variation in attractiveness of green light  $\lambda 5461 \text{ Å}$  with varying intensity (Method C). Abscissa, intensity of green,  $\mu w$ . Ordinate, ratio attracted by green when white is of equal intensity.

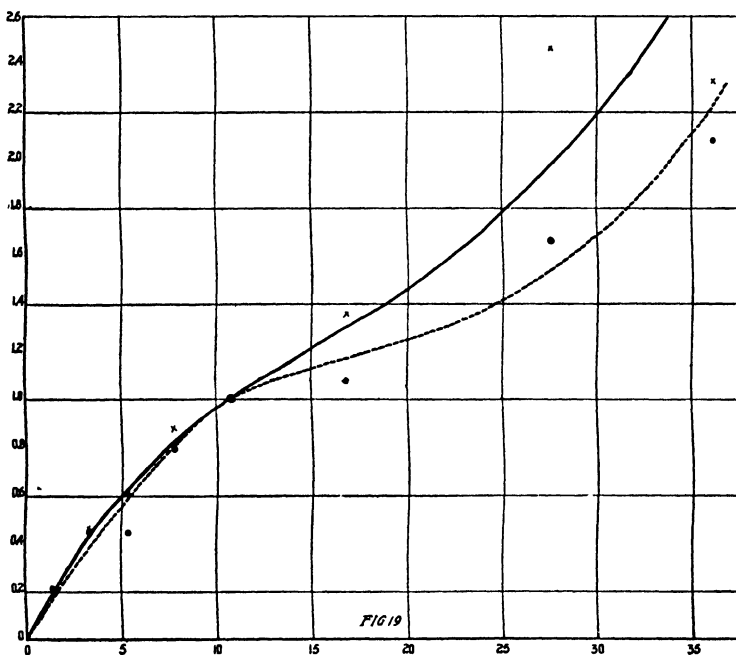
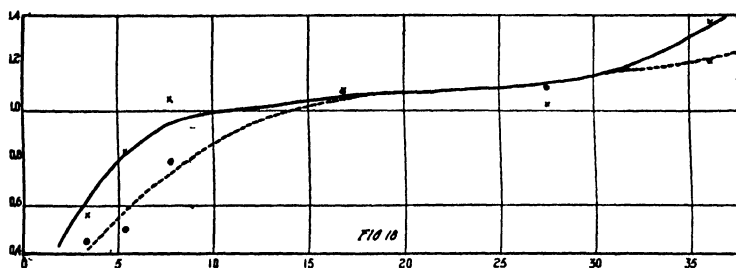
the value for relative stimulative effect, to give, presumably, the stimulative value per unit of energy. This method is not valid, since it is apparent from Bertholf's own results, as well as from Sander's, that the efficiency does not vary directly with the intensity. This is easily seen by referring to Bertholf's curve showing the response to two beams of white light of unequal intensity (5, Fig. 2). If the method were correct,  $R/I$  should be a constant,  $R$  being the relative response, and  $I$  the relative intensity. That this is not the



FIGS. 14-17. Abscissae, intensity of white light,  $\mu w$ . Ordinates, ratio attracted to white light. Males —X—, Females ---O---. FIG. 14. Relation between attractiveness of light  $\lambda 3022 \text{ \AA}$  at an intensity of 10.43 (for males) and 10.40  $\mu w$ . (for females), and white light of varying intensity. FIG. 15. Relation between attractiveness of light  $\lambda 3132 \text{ \AA}$  at an intensity of 10.09  $\mu w$ . (for both sexes), and white light of varying intensity. FIG. 16. Relation between attractiveness of light  $\lambda 3656 \text{ \AA}$  at an intensity of 10.78 (for males) and 10.59  $\mu w$ . (for females), and white light of varying intensity. FIG. 17. Relation between attractiveness of light  $\lambda 4078 \text{ \AA}$  at an intensity of 9.77 (for males) and 9.71  $\mu w$ . (for females), and white light of varying intensity.

case is easily demonstrated. For example, when  $I = 2.2$ ,  $R = 1.4$ , and  $R/I = 0.64$ ; when  $I = 1.1$ ,  $R = 1.05$ , and  $R/I = 0.95$ ; and similarly for other values.

Furthermore, this curve cannot be accepted as showing the response to lights of various *relative* intensities, but is correct only for the particular fixed intensity of white used. This can be seen by referring to Bertholf's data (5,



FIGS. 18-19. Abscissae, intensity of white light,  $\mu w$ . Ordinates, ratio attracted to white light. Males — $\times$ —. Females --- $\circ$ ---. FIG. 18. Relation between attractiveness of light  $\lambda 4359 \text{ \AA}$  at an intensity of 9.87 (for males) and 9.78  $\mu w$ . (for females), and white light of varying intensity. FIG. 19. Relation between the attractiveness of two white lights of different intensity. Intensity of standard white 10.78  $\mu w$ . (for both sexes).

Table II). Consider that the fixed light is of the intensity 2.98. Then for a relative intensity of the variable light of 0.335 (*i.e.*, 1.0 is now the intensity of the variable light, and is of the intensity ratio 2.98 : 1.0, or 1.0 : 0.335) the relative response would plot as 0.6 (reciprocal of 1.67), whereas the value read from the curve for this intensity ratio is 0.73. Similarly, if the standard

had been of the intensity represented by 0.5, at a relative intensity of 2.0 in the variable light the relative response would have been 1.17 instead of 1.35.

Sander doubts the validity of Bertholf's correction calculations, and in view of the foregoing discussion, some consideration may be given to the question at this time. Firstly, the two curves are united by multiplying the values of the second series by the factor 5206.7. The line 436  $m\mu$  obtained from the quartz-mercury arc in this series was of much greater intensity

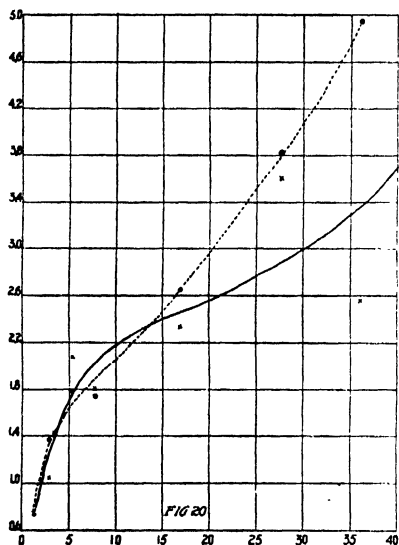


FIG. 20. Abscissa, intensity of white light,  $\mu v$ . Ordinate, ratio attracted to white light. Males —x—. Females —o—. Relation between attractiveness of light  $\lambda 5780 \text{ \AA}$  at an intensity of 9.97 (for males) and 9.98  $\mu v$ . (for females), and white light of varying intensity

than the 431  $m\mu$  obtained from the projection bulb in the first series. The "relative" energies given in the two cases are 78.1 and 0.18 respectively, though no clue is given as to the value of 1.0, or whether it is the same in both cases. Later it will be shown from the data of this study that, according to the method of analysis used by Bertholf, the relative stimulative efficiency apparently increases as the intensity of the light decreases. Hence, had  $\lambda 436 m\mu$  been of an intensity comparable to that of  $\lambda 431 m\mu$ , it is probable that the factor for joining the two curves would have been much smaller. This would have lowered very materially the peak shown at  $\lambda 365 m\mu$ . Secondly, relative intensity 1.0 of the standard was obtained from a 10-watt lamp in Bertholf's second series of experiments, while in the first series the source was a 100-watt lamp, so that the two sets of results cannot be compared.

One great difficulty throughout these papers is that the intensity of white is always expressed as a ratio of white, and the intensity of color as a ratio of color, with no clue as to what ratio 1.0 represents in units of energy, and consequently no means of ascertaining the relation between the intensity of the standard and the test lights. In the discussion of the second series the statement is made (6, p. 716) that "the minimum intensity 10 equals approximately 3.5 meter-candles." This, however, is a visual unit, and hence is unsuitable for the comparison of intensity of colors.

Analysis of Sander's (88) figures from his first series of experiments (Method C) shows that neither does this method give results that are truly indicative of the relative stimulative efficiency of colored lights. In this series all the lights were of equal energy when tested against each other. From his Table III, when  $\lambda 610 m\mu$  was used as a standard,  $\lambda 530 m\mu$  attracted 64.7% of the total number of bees reacting. That is, the ratio of attractiveness of

$\lambda 530 \text{ m}\mu$  was 64.7/35.3, or 1.833. If the relative stimulative efficiencies are in the ratio of the numbers attracted by lights of equal intensity, then  $\lambda 530 \text{ m}\mu$  is 1.833 times as efficient as is  $\lambda 610 \text{ m}\mu$ . That being so, increasing the intensity of  $\lambda 610 \text{ m}\mu$  to 1.833 times that of  $\lambda 530 \text{ m}\mu$  should make them equal in attractiveness. However, in the second series of tests (Method D), when these two wave-lengths were each tested against  $\lambda 570 \text{ m}\mu$ , it was found that  $\lambda 610 \text{ m}\mu$  had to be increased to 341.1%, and  $\lambda 530 \text{ m}\mu$  to 164.4%, of the intensity of  $\lambda 570 \text{ m}\mu$ , in order to be equally attractive. When subsequently tested together at these higher intensities, the two lights were found to be equal to each other in attractiveness. That is,  $\lambda 610 \text{ m}\mu$  had to be 341.1/164.4, or 2.077, times as strong as  $\lambda 530 \text{ m}\mu$  in order to be equally attractive. It would seem then that this is the true relative value of the two lights. Apparently neither intensity nor wave-length influenced the results obtained by this method. Other groups of wave-lengths, such as  $\lambda 570 \text{ m}\mu$ ,  $\lambda 490 \text{ m}\mu$ , and  $\lambda 365 \text{ m}\mu$ , were used with the same results. In some tests the comparison light was left at constant intensity and each of the test lights was varied in intensity until it attracted the same number of bees as did the standard. In other tests, the comparison light was changed in intensity until it was of the same attractiveness as one of the test lights, and the second test light was then made as attractive as the new intensity of the standard. The results obtained were equally good by the two methods, only minor changes, such as would be expected on the basis of experimental error, being necessary in order to make the two test lights equal in attractiveness. It is unfortunate that each group of wave-lengths was not tested at several different intensities of the standard, in order to determine whether the relation would remain constant. This would be the final test of the validity of the method.

In discussing the merits of the two methods outlined in the foregoing, Mast (66) says: "Sander maintains that the relation between stimulating efficiency and energy varies with wave-length, and that Bertholf's method is consequently inadequate. It may also be said that the difference in the stimulating efficiency of two lights is not proportional to the frequency of selection and that Sander's method is consequently also inadequate."

Mast (*in litt.*) has enlarged on this as follows: According to Sander's method, if two light sources are equal in energy, then their stimulating efficiency is directly proportional to the number of insects that go toward them; for example, if twice as many go toward *A* as toward *B*, the stimulating efficiency of *A* is twice as great as that of *B*. But if all the insects go toward *A*, it would mean that the stimulating efficiency of *A* is infinite and that of *B* zero, which usually is not true, as demonstrated by observations on *Eristalis*. Mast thinks it merely shows that the stimulating efficiency of *A* is greater than that of *B*, but not how much greater.

This argument may be partially answered as follows: Suppose that light *A* is the standard and that *B* is the wave-length under investigation. Then if *A* and *B* are of equal intensity and all the flies go toward *A*, it does actually mean that the stimulative efficiency of *B* is zero relative to *A*; and if a curve

for efficiency throughout the spectrum were drawn, it would reach zero at the point on the wave-length scale corresponding to *B*, exactly as Bertholf found it to do in the red end of the spectrum for the honeybee. On the other hand, if *A* is being used as a standard for testing relative attractiveness, it must of necessity have some attractiveness for the insects. Consequently, if *A* and *B* are equal in intensity, it does not seem probable that *B* would attract all the insects, and therefore the numbers attracted might be a measure of the relative efficiencies of *A* and *B*. Further consideration is given to this point in connection with the results of the present experiments.

Owing to the variation in the results obtained by these different methods, it was considered advisable to examine critically the method of testing and the analysis of the data in these experiments. In order to do this, all methods have been applied, as far as possible, to the data obtained. To show the part that intensity plays in determining the response to light,  $\lambda 5461 \text{ \AA}$  was tested at five, and  $\lambda 4078 \text{ \AA}$  at three, different intensities. The weakest green line was of such low intensity that the curve (Fig. 8) showing its effect is not as complete as for the other intensities, and therefore is not included in some of the analyses. The effect of intensity is considered first.

#### *Analysis of results with different intensities of color by Method A*

This is the method that Bertholf (6) used in testing the response of the honeybee to different wave-lengths of ultraviolet. The results obtained in tests with green light on the housefly are shown in Table V.

TABLE V

STIMULATIVE EFFICIENCY OF DIFFERENT INTENSITIES OF  $\lambda 5461 \text{ \AA}$  FOR *M. domestica*, AS DETERMINED BY METHOD A

Intensity of standard white,  $10.78 \mu\text{w}$ .

	Intensity of green, $\mu\text{w}$ .	Intensity of equally attractive white, $\mu\text{w}$ .	Relative stimulative effect	Relative energy of green	Relative stimulative efficiency
Male	21.90	3.30	0.306	2.121	0.1442
	14.42	1.06	0.0984	1.397	0.0704
	7.94	0.97	0.0900	0.769	0.1170
	5.96	0.45	0.0418	0.577	0.0725
	4.10	0.42	0.0390	0.397	0.0982
Female	21.47	8.20	0.7608	2.080	0.366
	14.80	1.30	0.1206	1.434	0.084
	7.94	1.28	0.1188	0.769	0.154
	5.98	1.47	0.1365	0.579	0.236
	4.12	0.90	0.0835	0.399	0.209

The intensity of white light equally as stimulative as the given intensity of green (ratio of attractiveness 1.0), is found from the curves showing the relative responses (Figs. 4 to 8). For comparative purposes these intensities

are plotted in Fig. 12. In order to express this as the "relative stimulative effect", it is divided by 10.78, the intensity of the standard white light (Table II). Bertholf expressed intensity of white light as an arbitrary scale, in which "the minimum intensity 10" (later changed to 0.1 in the graphs) "equals approximately 3.5 metre-candles". Consequently, the column showing intensity in absolute units is not found in his table. The "relative" results obtained by the two methods, however, will be the same. The "relative energy" of green is expressed as its ratio of 10.32 (the strength of green chosen for comparison with the other colors, as previously explained), and the "relative stimulative efficiency" is obtained by dividing these values into those for relative effect. The results obtained by this method of analysis are irregular, so that it is not possible to draw any definite conclusions. It may be noted that at the highest intensity of green tested, the relative stimulative efficiency for the males (Table V) is definitely higher than at any other intensity. The lowest intensity shows a relative efficiency of approximately two-thirds as much, while that of the middle intensity lies between these two. The remaining two intensities appear to be considerably lower in relative stimulative efficiency. Results with the females (Table V) show a similar irregularity, but the trend is the same, the higher intensity again appearing to be definitely better in relative stimulative efficiency. The irregularities may be explained on the basis of experimental error, but in any case the results vary sufficiently to show that this method cannot be used for comparing lights of different wave-lengths, unless they are of equal energy content.

Analysis of results with violet light by this method (Figs. 9 to 11, and Table VI) also shows irregularity, but here the lowest intensity tested appears to have the greatest stimulative value. An interesting point may be noted from these results, *viz.*, that for males (Table VI) the intermediate intensity of 6.75  $\mu\text{w.}$  is equalled in attractiveness by white of 5.80  $\mu\text{w.}$ , while violet of only

TABLE VI  
STIMULATIVE EFFICIENCY OF DIFFERENT INTENSITIES OF  $\lambda 4078 \text{ \AA}$  FOR *M. domestica*, AS  
DETERMINED BY METHOD A  
Intensity of standard white, 10.78  $\mu\text{w.}$

	Intensity of violet, $\mu\text{w.}$	Intensity of equally attractive white, $\mu\text{w.}$	Relative stimulative effect	Relative energy of violet	Relative stimulative efficiency
Male	9.77 6.75 4.41	18.55 5.80 11.00	1.721 0.538 1.020	1.000 0.691 0.451	1.721 0.778 2.262
Female	9.71 6.76 4.12	27.50 8.60 18.30	2.551 0.798 1.697	1.000 0.696 0.424	2.551 1.147 4.005

4.41  $\mu\text{w.}$  requires white of 11.00  $\mu\text{w.}$  to be equally attractive. A similar result is obtained with the females. The distribution of the data (Figs. 10 and 11) appears to indicate that this is a real effect. If so, the usually accepted theory, that lights become more attractive as they become more intense, will have to be modified somewhat. Further investigation of this point is indicated.

*Analysis of results with different intensities of color by Method B*

In this method of analysis all the colors are tested against the same intensity of white. The standard intensity is 10.78  $\mu\text{w.}$ , but as none of the different wave-lengths were actually tested against this intensity, values are interpolated from the graphs (Figs. 4 to 11), and the results with different intensities of green are shown in Table VII. The first column shows the absolute

TABLE VII

STIMULATIVE EFFICIENCY OF DIFFERENT INTENSITIES OF  $\lambda 5461 \text{ \AA}$  FOR *M. domestica*, AS DETERMINED BY METHOD B

Intensity of standard white, 10.78  $\mu\text{w.}$

	Intensity of green, $\mu\text{w.}$	Relative stimulative effect	Corresponding intensity of white, $\mu\text{w.}$	Relative stimulative value	Relative energy of green	Relative stimulative efficiency
Male	21.90	0.693	6.03	0.560	2.121	0.264
	14.42	0.786	7.18	0.666	1.397	0.477
	7.94	0.638	5.40	0.501	0.769	0.652
Female	21.47	0.885	8.79	0.816	2.080	0.392
	14.80	0.794	7.62	0.707	1.434	0.493
	7.94	0.862	8.45	0.784	0.769	1.019

values of the three intensities of green used. The second column shows the relative stimulative effect, which is the ratio between the numbers attracted to green and to white (the reciprocals of the values determined from the graphs). The third column is obtained from Fig. 19, and is the intensity of white necessary to give this ratio of attractiveness when tested against the standard white. This column is not found in Bertholf's table, as he did not use absolute values. The numbers in the fourth column are obtained by dividing those of the third by 10.78 and correspond to Bertholf's "relative stimulative value", except that the intensity that he used as unity is not indicated. Relative energy of green is obtained by dividing the values of the first column by 10.32. Finally, relative stimulative efficiency is obtained by dividing the values of the fifth column into those of the fourth. The results show clearly that this method of analysis is not adequate, as the final value found depends on the intensity of green used and decreases markedly as the intensity increases. The result is thus exactly the reverse of that obtained by Method A, by which it was found that stimulative efficiency apparently increases as the intensity

increases. That this variation of efficiency is real and not accidental is shown by Table VIII. This shows the results of a similar analysis of the data obtained using three different intensities of violet light of  $\lambda 4078 \text{ \AA}$  (Figs. 9 to 11). The results are practically the same, though those with the females are somewhat irregular. However, with this method as with Method A, there seems to be distinct evidence that a decrease in the intensity of this color results in a great increase in its relative stimulative efficiency. Consequently, different wave-lengths cannot be compared by this method unless they are of equal intensity. For example, had the results with green at the intensity of  $21.90 \mu\text{w.}$  been used in constructing the curve in Fig. 21, it would have indicated that this color was only about one-fourth as attractive as violet of intensity  $9.77 \mu\text{w.}$  (relative efficiencies 0.264 and 1.055 respectively, Tables VII and VIII). Actually, when tested at approximately the same intensity, green is found to be more than half as attractive as violet (0.6 compared with 1.055).

TABLE VIII

STIMULATIVE EFFICIENCY OF DIFFERENT INTENSITIES OF  $\lambda 4078 \text{ \AA}$  FOR *M. domestica*, AS DETERMINED BY METHOD B

Intensity of standard white,  $10.78 \mu\text{w.}$

	Intensity of violet, $\mu\text{w.}$	Relative stimulative effect	Corresponding intensity of white, $\mu\text{w.}$	Relative stimulative value	Relative energy of violet	Relative stimulative efficiency
Male	9.77	1.037	11.36	1.055	1.000	1.055
	6.75	0.883	8.54	0.792	0.691	1.146
	4.41	1.012	10.80	1.002	0.451	2.221
Female	9.71	1.199	17.70	1.642	1.000	1.642
	6.76	0.893	8.92	0.828	0.696	1.190
	4.12	1.117	14.18	1.315	0.424	3.101

The effect of calculation on the final results obtained by the two methods is worthy of note. In the violet, the relative stimulative effect for both sexes by either method is less for the intermediate than for the lowest intensity. -By Method A, this is found also in the final value for relative stimulative efficiency (Table VI). By Method B (Table VIII), irregularity is not found in the results with the males, the relative efficiency apparently increasing as the intensity decreases. For the females the irregularity is much less than that obtained by Method A. As the same data are used in both cases, these differences in results are attributable only to the calculations involved. This makes it quite evident that one or both of these methods are inadequate for the analysis of data of this type.

#### *Analysis of results with different intensities of color by Method C*

The results obtained for green by this method are tabulated in Table IX. For each intensity of green there is found from the graphs (Figs. 4 to 7) the

ratio attracted by white of equal intensity, and the reciprocal of this then gives the ratio attracted by green. The results are plotted in Fig. 13. Again it is seen that there is a great difference in relative attractiveness as the in-

TABLE IX

STIMULATIVE EFFICIENCY OF DIFFERENT INTENSITIES  
OF  $\lambda 5461 \text{ \AA}$  FOR *M. domestica* AS DETERMINED  
BY METHOD C

	Intensity of green, $\mu\text{w.}$	Ratio attracted by white of equal intensity	Ratio attracted by green
Male	21.90	1.623	0.616
	14.42	1.300	0.769
	7.94	1.485	0.673
	5.96	1.595	0.627
Female	21.47	1.448	0.691
	14.80	1.423	0.703
	7.94	1.112	0.899
	5.98	1.282	0.780

TABLE X

STIMULATIVE EFFICIENCY OF DIFFERENT INTENSITIES  
OF  $\lambda 4078 \text{ \AA}$  FOR *M. domestica* AS DETERMINED  
BY METHOD C

	Intensity of violet, $\mu\text{w.}$	Ratio attracted by white of equal intensity	Ratio attracted by violet
Male	9.77	0.956	1.046
	6.75	1.036	0.965
	4.41	0.544	1.828
Female	9.71	0.818	1.222
	6.76	0.881	1.135
	4.12	0.772	1.296

tensity of the green varies. Apparently the efficiency of this wave-length increases quite markedly as the intensity increases, up to a certain point and then it decreases. There is a very evident difference between the sexes as to the point at which maximum stimulation is found.

Examining the results obtained with different intensities of violet light ( $4078 \text{ \AA}$ ), found from Figs. 9 to 11, and shown in Table X, it again becomes evident that intensity plays an important part, although the females show less pronounced differences with this color than with green. Nevertheless, results for both sexes seem to show that lower intensities are more stimulative than higher, when this method of analysis is used.

Referring again to the statement (p. 327) regarding the attractiveness of the intermediate intensity of violet, it may be noted that, in spite of the widely different numerical values obtained, all three methods of analysis show for

the females, and two for the males, this phenomenon of decreased efficiency. In some cases the difference is not great, and possibly further work will show that it is merely experimental error. It is generally assumed that as the intensity of light increases, its attractiveness increases up to a certain point, and beyond this there may be a decrease in attractiveness or an actual repellency. Should the foregoing results prove to be well founded, it would indicate that  $\lambda 4078 \text{ \AA}$ , as it increases in intensity over the range here tested, becomes at first less, and later more, attractive to *M. domestica*.

*Analysis of results with different colors of approximately equal intensity*

Tables XI to XIII show the analyses of the final results for all colors with which *M. domestica* was tested, using the three methods described above.

TABLE XI

STIMULATIVE EFFICIENCY OF DIFFERENT WAVE-LENGTHS, APPROXIMATELY EQUAL IN INTENSITY, FOR *M. domestica*, AS DETERMINED BY METHOD A

Intensity of standard white, 10.78  $\mu$ w.

	Wave-length, Å	Intensity of equally attractive white, $\mu$ w.	Relative stimulative effect	Relative energy of color	Relative stimulative efficiency
Male	3022	7.70	0.714	0.968	0.738
	3132	15.50	1.439	0.936	1.537
	3656	45.20	4.194	1.000	4.194
	4078	18.55	1.721	0.907	1.898
	4359	10.50	0.994	0.916	1.085
	5461	0.94*	0.087	0.956	0.091
	5780	2.10	0.195	0.925	0.211
Female	3022	12.73	1.181	0.965	1.224
	3132	24.80	2.301	0.936	2.459
	3656	56.90	5.280	0.982	5.377
	4078	27.35	2.538	0.901	2.817
	4359	14.15	1.313	0.907	1.447
	5461	1.58*	0.147	0.958	0.153
	5780	1.76	0.163	0.926	0.176

\* From Fig. 12.

TABLE XII

STIMULATIVE EFFICIENCY OF DIFFERENT WAVE-LENGTHS, APPROXIMATELY EQUAL IN INTENSITY, FOR *M. domestica*, AS DETERMINED BY METHOD B

Intensity of standard white, 10.78  $\mu$ w.

	Wave-length, Å	Relative stimulative effect	Correspond- ing intensity of white, $\mu$ w.	Relative stimulative value	Relative energy of color	Relative stimulative efficiency
Male	3022	0.952	9.80	0.909	0.968	0.939
	3132	1.052	11.92	1.106	0.936	1.182
	3656	2.000	27.84	2.583	1.000	2.583
	4078	1.037	11.36	1.055	0.907	1.163
	4359	0.997	10.67	0.990	0.916	1.081
	5461	0.708*	6.23	0.578	0.956	0.604
	5780	0.451	3.52	0.327	0.925	0.353
Female	3022	1.078	12.82	1.190	0.965	1.233
	3132	1.465	26.00	2.411	0.936	2.576
	3656	2.433	37.65	3.492	0.982	3.556
	4078	1.193	17.42	1.616	0.901	1.793
	4359	1.111	14.00	1.299	0.907	1.432
	5461	0.832*	8.03	0.745	0.958	0.778
	5780	0.463	4.12	0.381	0.926	0.411

\* From Fig. 13.

In Table XIII (Method C), in order to make the results comparable, it is assumed that the intensity of each of the spectral colors was the same as that of the standard white, *viz.*, 10.78  $\mu\text{w.}$ ; the values given are found by interpolation in Figs. 14 to 20 for the colors tested against this intensity of white.

TABLE XIII  
STIMULATIVE EFFICIENCY OF DIFFERENT WAVE-LENGTHS,  
APPROXIMATELY EQUAL IN INTENSITY, FOR  
*M. domestica*, AS DETERMINED BY METHOD C  
Intensity of standard white, 10.78  $\mu\text{w.}$

	Wave-length, $\text{\AA}$	Ratio attracted by white	Ratio attracted by color
Male	3022	1.050	0.952
	3132	0.950	1.052
	3656	0.500	2.000
	4078	0.964	1.037
	4359	1.002	0.997
	5461	—	0.713*
	5780	2.219	0.451
Female	3022	0.928	1.078
	3132	0.683	1.465
	3656	0.411	2.433
	4078	0.838	1.193
	4359	0.900	1.111
	5461	—	0.818*
	5780	2.160	0.463

\* From Fig. 13.

$\text{\AA}$ , with a rapid decrease on both sides of this wave-length. Quantitatively, none of the methods appears to be satisfactory, because, by varying the intensity of either test or standard light, the values for relative efficiency can be changed within quite wide limits, as shown in Tables V to X. It is unfortunate that the equipment available was not adaptable to testing reactions by Method D, already described. By this method, Sander has shown that lights that are made equal in attractiveness to a common standard are equal to each other. Hence it would appear that the true relative stimulative efficiencies are indicated by the intensities required to give this equal attractiveness.

In the tests in which all the colored lights were of equal intensities, the final values obtained by Method C (Table XIII, Column 3) are the same as the values for relative stimulative effect found by Method B (Table XII, Column 2). This being so, the difference in the final results obtained by the two methods must be due to the calculations used in Method B. If the colored and standard lights are of unequal energy, no relation is found between the two methods.

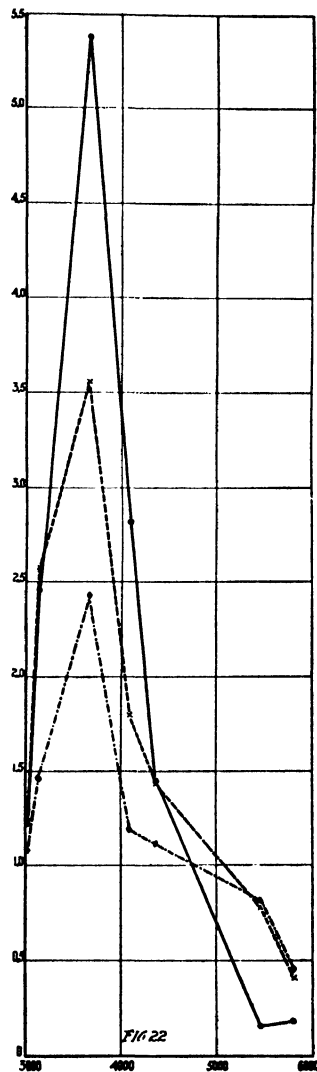
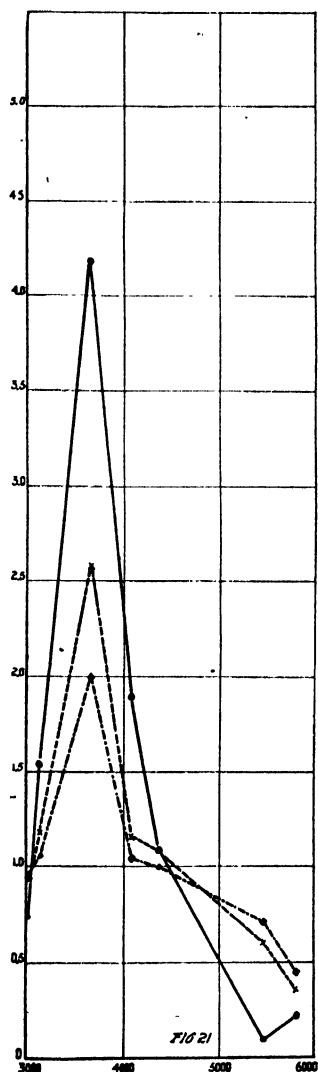
If Method B gave the true relative efficiency values, then the values in the second column of Table XI should be the same as those in the third column of Table XII, respectively. That is,  $\lambda 3022 \text{ \AA}$  of intensity 10.43  $\mu\text{w.}$  attracts

This assumption is justified in view of the fact that the greatest variation between the intensities of the different colors is not over 10%, estimated as the approximate limit of error in the measurements (p. 314). As green was not tested at this intensity, the values required for this color are read directly from Fig. 12 or 13, as indicated in the tables. For a better comparison, the results obtained by all three methods are plotted for each sex in Figs. 21 and 22.

### Discussion

The three methods of analysis give practically identical results qualitatively. That is, all show the peak of attractiveness to be at  $\lambda 3656$

0.952 times as many male flies as does white of intensity  $10.78 \mu\text{w.}$ , and white of intensity  $9.80 \mu\text{w.}$  also attracts 0.952 times as many male flies as does the standard white (from Table XII). Therefore the test light  $\lambda 3022 \text{ \AA}$  should be equal in attractiveness to white of intensity  $9.80 \mu\text{w.}$  But from Table XI it is seen that light of this wave-length at intensity  $10.43 \mu\text{w.}$  is actually equalled by white of intensity  $7.70 \mu\text{w.}$ ; that is, the lights are not equal when they attract the same fraction of the number of flies attracted by the standard intensity of white. Results for the other wave-lengths are similar, some showing even less agreement, and some, as  $\lambda 4359 \text{ \AA}$  for the



FIGS. 21-22. Abscissae, wave-length. Ordinates, relative stimulative efficiency. Method A ————C Method B ————X———, Method C ————◇———. FIG. 21. Relative attractiveness of lights of equal intensity but different wave-length, as determined by Methods A, B, and C, for males. FIG. 22. Same for females.

males, having almost identical values. It will be seen, though, that here the attractiveness of the color and of the standard white were almost equal (ratio 1.0 : 0.997, Table XII). Similarly in the other instances in which results are in close agreement, the ratio of attractiveness at the intensities tested is not far from 1.0. This further suggests that Method D is the best criterion of comparative attractiveness.

Method C shows much less pronounced differences between the different wave-lengths than do A or B, Method A giving an especially steep curve in Figs. 20 and 21. The latter method shows an apparent increase in attractiveness for both males and females, from green to yellow, and this is more pronounced for the males. Unfortunately, the arc lamp did not have sufficient energy in the orange and red lines to continue the curve on a comparable intensity basis into the long-wave region, and the monochromator did not give a sufficiently narrow band from an incandescent bulb to use this as a source. Consequently, it is not possible at present to say whether this rise would continue as the curve is carried further into the long-wave region. As the other methods show yellow to be considerably less efficient than green, it would seem that this apparent rise is merely a result of the calculations required by Method A, rather than a real increase in efficiency.

It is obvious that results obtained by one method cannot be compared with those obtained by any other. This is especially true when the intensities of the various colored lights are unequal. It also is obvious that curves obtained by different methods cannot be joined by calculation, as was done by Bertholf. Inasmuch as Sander actually tested lights of equal intensity but different wave-length against each other and drew his curves for relative stimulative efficiency from the data so obtained, it appears that, for the time being, this curve should be taken as the most nearly correct qualitative representation of the efficiency of different wave-lengths in attracting the honeybee.

Similarly, the curve derived by Method C from the data here obtained in tests with houseflies probably gives the most nearly correct picture of relative attractiveness of different spectral lights for this insect. It has already been pointed out that even this method does not show the true quantitative relation between the colors. That still remains to be determined.

It had previously been found by Hess (44) that for the housefly, as well as for other insects, the most stimulative region was in the blue and green, which were much more attractive than yellow and red. This is entirely in accordance with the results found here, except that the longer wave-lengths of ultraviolet, which Hess did not test, are found to be most attractive of all.

On the other hand, Freeborn and Berry (27) carried out tests in which a board with painted squares ranging in color from blue to red, and including white and aluminium, was exposed to houseflies in a dairy barn for three months. Evaluation of the attractiveness by means of the dots of excrement on the squares showed that dark blue was the most attractive, followed by dark red, light gray and canary yellow. There was then a slight break in the continuity, and a median range of attractiveness was found to consist of

orange, aluminium, jade green and light blue. A second much sharper break followed, and in the least attractive group were white, coral, foam green, ivory and primrose. Unfortunately there are no data as to the spectral range of the light reflected by these various paints. The names, of course, give a rough indication of their range in the visible spectrum, but some or all of them might reflect ultraviolet as well. This latter would under the circumstances of the experiment be a relatively minor factor, because the board was fastened to the ceiling of the barn and consequently little ultraviolet would reach it in the daytime. But at night the board was lighted by three 100-watt "daylight" lamps, and these do emit a certain amount of ultraviolet, especially of the longer wave-lengths. It is possible that the red did reflect some of this ultraviolet, which might account for its very high attractiveness, or it may be that the red itself is actually highly attractive. In this latter case there is justification for Kell (51), who has patented a fly paper "having a color for which the fly exhibits a preference, e.g., dark blue or dark red". However, Pilkington Bros. (81) report that the use of red and yellow glass in windows greatly reduced the housefly population in buildings, while blue and green were not nearly as effective. They believed this to be due to a deterrent effect of the red and yellow, discouraging entry of the flies. Apparently the factor of intensity was not considered, and it may have contributed somewhat to the results obtained.

Figs. 21 and 22 show that at all wave-lengths tested the females of *M. domestica* are more strongly stimulated by colored lights than are the males. This can be seen also by examination of the graphs for each individual wave-length (Figs. 4 to 20). Here, in nearly every case, more males than females are attracted by the white at any given intensity, except in the intensity range in which the color and white are almost equal in attractiveness. In this range there is little difference between the responses of the two sexes. Again the relative differences vary according to the method of analysis chosen, Method C showing the least difference between the two sexes. The peak of attractiveness at  $\lambda 3656 \text{ \AA}$  is considerably higher for females than for males, but the rate of decline from this peak is correspondingly rapid on both sides. At  $\lambda 5780 \text{ \AA}$ , attractiveness appears to be about equal for the two sexes. The rise is very rapid from  $\lambda 4078 \text{ \AA}$  to  $\lambda 3656 \text{ \AA}$ , the curve resembling that found by Berthöf (6) for the honeybee but differing from that found by Sander for the same insect. It is possible that the line connecting  $\lambda 4359 \text{ \AA}$  and  $\lambda 5461 \text{ \AA}$  in Figs. 21 and 22 would be curved instead of straight, had there been a spectral line of sufficient intensity at about  $\lambda 4900 \text{ \AA}$  available for testing. It would have been interesting to have seen whether the rapid decrease in effectiveness at the short wave-lengths continued to zero as the limit of the ordinary sunlight spectrum was reached, or whether the flies were able to detect radiation even beyond this, as was found by Berthöf (7) with *Drosophila*. These are problems that remain to be solved.

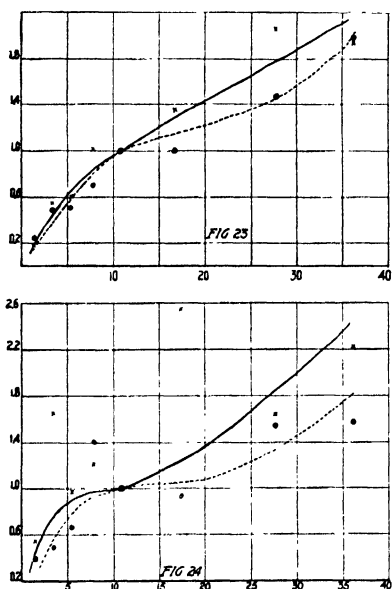
All curves for the various wave-lengths appear to have a characteristic sigmoid shape. That is, in all graphs where the data are sufficient, the curve

has three definite regions. First there is a region in which the ratio of flies going to white is quite definitely less than 1.0. This part of the curve is quite steep, as a comparatively small increase in the intensity of the comparison white light causes a relatively large change in its attractiveness. There is a second region, in which the lights are of approximately equal attractiveness and which is quite flat. This relation extends over a wide range of intensity of the white light and indicates that the fly does not distinguish clearly between the two lights. In the third region the curve again becomes steeper, and small changes in intensity have a great effect on attractiveness.

In other words, it appears that if one of two equally attractive lights is kept at a constant intensity, the intensity of the second may be varied over a considerable range before they become appreciably different in attractiveness to the housefly. When, however, this range has been exceeded in either direction, relatively small changes in intensity of the variable light cause marked changes in its attractiveness. This apparently is the type of reaction meant by Verworn (95), who stated that there is a threshold of stimulation below which the stimulus is ineffective, and above which the effect increases with increasing intensity of the stimulus.

The median range is considerably smaller in the curve for variable white *versus* constant white (Fig. 19) than in those curves in which the constant light is colored. An exception perhaps is that for yellow (Fig. 20). The similarity between these two curves may be due to the fact that the "white" light, being obtained from an incandescent bulb, contains a large proportion of yellow. Tables (45, p. 693) show that the sensation produced by the tungsten filament is divided thus: red, 48%; green, 41%; blue, 11%. Consequently, the response obtained by this light would be expected to approximate more closely to those of the long-wave than of the short-wave colors.

The attractiveness of  $\lambda 3656 \text{ \AA}$  for *M. domestica* is so great that the maximum available intensity of white, some three and one-half times that of the color, was not enough to attract the same number of flies as did the color. As explained earlier (p. 317), the lower intensities of white were not tested against this color.



FIGS. 23-24. Abscissae, intensity of variable white,  $\mu v$ . Ordinates, ratio attracted by variable white. Males — x —. Females — o —. FIG. 23. Relation between attractiveness of constant white light of intensity 10.78  $\mu v$ , and white light of varying intensity, using only first five responses of each fly. FIG. 24. Relation between attractiveness of constant white light of intensity 10.78  $\mu v$ , and white light of varying intensity, using only initial response of each fly.

Consequently, only a part of the median range of the curve is shown in Fig. 16.

Owing to the length of time each insect spends in the reaction box, there is a possibility that the eye will lose its dark adaptation somewhat, while the tests are being run. To determine if this is so, the data obtained from tests with two white lights were examined in two ways: (i) the first five responses of each fly were classified and tabulated (Table XIV); (ii) and the initial response of each fly was treated similarly (Table XV). The data for the two series

TABLE XIV

RESPONSE OF *M. domestica* TO WHITE LIGHTS OF UNEQUAL INTENSITY, COMPILED FROM FIRST FIVE RESPONSES OF EACH FLY

Intensity of standard white, 10.78  $\mu$ w.

	Intensity of variable white, $\mu$ w.	Number of responses	Number to standard white	Per cent to standard white	Ratio to variable white
Male	36.10	235	80	34.1	1.937
	27.66	190	62	32.6	2.066
	16.84	200	85	42.5	1.353
	7.73	240	119	49.6	1.016
	5.35	210	131	62.4	0.603
	3.30	220	142	64.6	0.550
	1.46	150	127	84.7	0.181
Female	36.10	230	77	33.5	1.987
	27.66	210	85	40.5	1.470
	16.84	220	110	50.0	1.000
	7.73	205	120	58.6	0.708
	5.35	210	140	66.7	0.500
	3.30	225	152	67.6	0.480
	1.46	175	141	80.6	0.241

TABLE XV

RESPONSE OF *M. domestica* TO WHITE LIGHTS OF UNEQUAL INTENSITY, COMPILED FROM INITIAL RESPONSE OF EACH FLY

Intensity of standard white, 10.78  $\mu$ w.

	Intensity of variable white, $\mu$ w.	Number of responses	Number to standard white	Per cent to standard white	Ratio to variable white
Male	36.10	55	17	30.9	2.235
	27.66	53	20	37.7	1.650
	16.84	56	22	39.3	2.547
	7.73	53	24	45.3	1.209
	5.35	53	27	50.9	0.963
	3.30	53	20	36.4	1.650
	1.46	37	24	64.9	0.542
Female	36.10	54	21	38.9	1.571
	27.66	51	20	39.2	1.550
	16.84	56	29	51.8	0.931
	7.73	53	22	41.5	1.409
	5.35	52	31	59.6	0.678
	3.30	52	35	67.3	0.486
	1.46	39	28	71.8	0.393

are plotted in Figs. 23 and 24 respectively. The two lights were not tested at equal intensities, but, as they were of the same quality, it may be assumed that if the intensities were equal the ratio of attractiveness would have been 1.0. Consequently, this point is shown on all curves.

The curve obtained using only the first five responses (Fig. 23) is remarkably like that for which ten responses of each fly were used (Fig. 19). When only the initial responses are included, the points are much more irregular. However, the number of observations is very much smaller, increasing the possibility of error. Considering this fact, the trend of the curve shown in Fig. 24 is sufficiently like that of Fig. 19 to indicate that the loss of dark adaptation during the tests is not sufficient to influence the results greatly.

Concerning the variation in selection of the contrasting lights by different flies, the question arises whether this is due merely to chance, or to variation in the ability of flies to distinguish color and brightness. If a fly is more attracted by one light than by another, then theoretically it should go to that light always and to the less attractive one not at all. Therefore, as the intensities vary, in a large number of trials there would be three conditions, *viz.*, a range in which all flies made all trips to the more attractive light, a median range in which the flies did not distinguish between the two lights and consequently 50% of a large number of trips would be to each, and finally a range in which all trips were to the second light, now more attractive. In other words, the behavior of the fly should indicate that the attractiveness of light A is more than, equal to, or less than that of light B. The fact that it is possible to draw a curve, to show the relative response as the intensity is varied by relatively small steps, indicates that there must be variation between individuals. That is, when two lights of different quality appear equal in attractiveness to one fly, one light may be more attractive to another fly.

The literature on the subject of variation in the ability of the human eye to distinguish differences in brightness is much too voluminous to discuss here, but Bertholf's (5) results may be cited. He found that when two light sources differed by 10% of the brighter, his subjects were able to distinguish the difference in only 62.5% of the trials. When the difference in the lights was 20% of the brighter, correct choices numbered 84.6% of the trials. This indicates that there is a variation between human eyes with regard to brightness discrimination.

There is also a difference with regard to the spectral limits of vision. It is usually considered that the human eye is unable to distinguish light of a wave-length shorter than about 4000 Å (29). De Groot (31) and Fabry (24), however, both give evidence to show that the line  $\lambda 3132$  Å of the mercury spectrum is visible to many, but not to all, people, while  $\lambda 3261$  Å is distinguished with ease. The writer and Dr. W. Rowles, Professor of Physics, Macdonald College, have made a few observations on this point, using a large spectrograph belonging to the Department of Physics. Both were able to distinguish the ultraviolet line of  $\lambda 3304$  Å without difficulty, and the writer could see  $\lambda 3132$  Å faintly. Suitable precautions were taken to insure that this was true vision, and not fluorescence in the eye.

As there is thus such wide variation between human eyes in their ability to distinguish both brightness and wave-length, it is conceivable that the eyes of insects might differ also. Therefore, the only logical way to determine the attractiveness of different wave-lengths of light for houseflies is to test a large population under a wide variety of conditions, and to average the data collected.

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## BACTERICIDAL AND ANTIGENIC QUALITIES OF THE WASHINGS OF BLOWFLY MAGGOTS<sup>1</sup>

BY RONALD GWATKIN<sup>2</sup> AND A. MURRAY FALLIS<sup>3</sup>

### Abstract

Washings of mixtures of maggots of *Calliphora erythrocephala*, *C. latifrons*, *C. vomitoria nigribarda* and *Cynomya cadaverina*, and of *C. erythrocephala* alone, showed bactericidal activity against *Staph. aureus*, *Strep. mastitidis*, two strains of *Brucella abortus*, and *B. typhosus in vitro*. Flies were successfully raised in the laboratory but washings from their maggots became less active with each generation. The pH value of active samples was generally greater than that of poor samples. Attempts to increase activity by rendering poor washings alkaline were unsuccessful. Diluted washings showed some bactericidal power. Keeping quality of liquid samples was poorer than that of dried ones. Washings from meat in which maggots had been grown had a definite bactericidal value, while those from control samples of meat had none. A suspension of maggots ground after washing was also inactive. Addition of *Br. abortus* to meat on which maggots were grown did not increase bactericidal activity against this organism. Filtration reduced bactericidal action and toxicity. Intraperitoneal injections were toxic to guinea pigs. An anti-maggot rabbit serum had good complement fixing power with an antigen of maggot washings. Intraperitoneal injections of diluted washings failed to protect guinea pigs against infection with *Br. abortus*, but there was some delay in development of infection, as indicated by slower appearance of agglutinins in treated animals.

### Introduction

Baer (1) in 1929, and in subsequent papers, reported on the successful use of blowfly maggots in surgical conditions.

Simmons (6) in 1935 obtained a bactericidal substance from the excretions of surgical maggots, *Lucilia sericata*. The active principle was not destroyed by autoclaving for 20 min. at 10 lb. pressure. Simmons points out that the perfection of a non-toxic, non-irritating disinfectant for internal use has not yet been realized and he suggests there are possibilities in this type of material.

Robinson (5) in 1935 reported the isolation of allantoin from maggot excretions. This substance is a product of metabolism of the cell nucleus of both plants and animals, and its excretion into wounds by the maggots is apparently one of the factors contributing to the healing effect in maggot therapy.

Stewart (7) in 1934 found that maggots of *L. sericata* exuded calcium carbonate. One hundred maggots produced an average of 0.6 mg. every 24 hr. He recommends treatment of osteomyelitis with calcium carbonate,

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picric acid, and glycerin. Frost and Errington (3) have found that this was beneficial in post-operative treatment of fistulous withers in horses.

It is curious to note, on the one hand, the beneficial effects of maggot therapy, and on the other, the harmful results produced by the same kind of maggots under natural conditions, an example of which is seen in the serious economic loss caused by blowflies in the sheep industry in Australia, Britain, and other sheep-raising countries. However, it is obvious that a substance possessing bactericidal qualities and capable of stimulating healing is of more than ordinary interest, if these qualities are not offset by others, such as toxicity, that might prevent its parenteral use.

## Methods and Materials

### METHOD OF RAISING FLIES

A culture of flies was established by attracting them to a dead rat or guinea pig, catching them in a net and enclosing them with pieces of raw beef in cheesecloth-covered glass jars. The flies that hatched from eggs laid on the meat provided stock cultures. The original flies were identified by Dr. C. H. Curran, Assistant Curator, Department of Entomology, New York Museum of Natural History. They were *Calliphora erythrocephala* Mg., *C. latifrons* Hough, *C. vomitoria nigribarda* S., and *Cynomya cadaverina* Desv.

The flies were kept in cages 2 ft. by 2 ft. by 2 ft., enclosed by cheesecloth, in a room about 19° C. The temperature was frequently lower and occasionally higher. The flies were fed on sugar and fresh meat. Water was supplied by inversion of a beaker on a Petri dish covered with a filter paper. An electric light close to the cages appeared to increase activity of the flies.

Larvae for experimental purposes were obtained by leaving a dish of meat in a cage of flies for several hours. When the meat was well "blown", the dish was removed to a glass jar that was covered with cheesecloth. The meat was covered with moist absorbent cotton to keep it from drying, as room humidity was low. After one or more washings to collect the experimental material, the larvae were allowed to pupate in dry sand.

The time intervals between the various stages of *C. erythrocephala*, although showing considerable variation due to inconstant temperature, were roughly as follows: eggs hatched in from 10 to 20 hr.; larval period lasted four to six days; prepupal period was one to three days, and the pupal stage about two weeks. The flies after emerging from the pupae were not ready to deposit eggs until a week or more later.

A relatively high humidity appeared to be favorable for all pre-adult stages, but the metamorphosis into pupae appeared to be favored by relatively dry conditions. Insufficient humidity during the pupal stage causes hardening of the cases and may prevent emergence of the flies.

### PREPARATION OF EXPERIMENTAL MATERIAL

Maggots were removed from the meat about four days after hatching and placed in an 80-mesh sieve on which another sieve was fitted to prevent their

escape. Removal of maggots was facilitated by using a relatively small amount of meat. Saline solution (0.85% NaCl) was dropped on them at regular intervals over a period of two hours, until a quantity had been used equal to one-half the volume of the maggots. The washings thus obtained were a dark green, or brownish-green, turbid liquid. This was autoclaved at 10 lb. pressure for 20 min., tested for sterility and stored in the refrigerator. Dried samples were not autoclaved until made into suspensions for use.

### BACTERICIDAL TESTS

Bactericidal tests were carried out after the manner of phenol coefficient tests. The procedure was similar to the method of Simmons (6). The results confirm those obtained by Simmons, although different kinds of bacteria were used as well as different species of maggots. Quantities of 2.5 cc. of sterilized washings were placed in test tubes in a water bath at 37° C. Twenty-four-hour broth cultures of bacteria were employed, 0.05 cc. of which was added to each tube at one-half minute intervals and also to the same quantity of salt solution for each culture against which the suspension was being tested. A standard loopful was removed from each tube of washings at 2.5, 5, 15, 30, and sometimes at 60 min. Transfers from the saline tubes were usually made after the conclusion of the experiment, but in some tests were made at the same time-intervals as the suspension under test. The broth tubes to which transfers had been made were incubated for 48 hr. at 37° C. *Staphylococcus aureus*, *Streptococcus mastitidis*, *B. typhosus* and two strains of *Br. abortus* were used at various times. There was a marked difference in resistance of the two *Brucella* strains. No. 10, an old laboratory strain, was much more easily killed than a more recently isolated culture. Suspensions were usually tested the day they were prepared, but in a few instances on the following day.

### Results

#### BACTERICIDAL ACTIVITY OF FRESH SAMPLES

Washings were obtained from 16 lots of maggots. Most of the lots were made up of more than one species of larvae, but some were prepared from the pure strain of *C. erythrocephala*. The source of the maggots, and the effect of the washings on the bacteria against which they were tried, are shown in Table I.

It will be noticed that better results were obtained from the early lots than from those produced from larvae of laboratory-raised flies. We have been unable to determine the cause of this. It was thought that crowding the maggots during the washing process might have been responsible, as a large quantity of maggots was placed in the sieve in the later experiments, completely filling it. This possibility was investigated with Lots 13 and 13A, which consisted of larvae from the same meat divided into small and large lots. There was no difference in bactericidal activity, but the larger lot had a slightly lower pH value. Mr. W. C. Henry, of the Department of Biochemistry, determined the pH of the maggot washings and saline solution

TABLE I  
RESULTS OF BACTERICIDAL TESTS OF MAGGOT WASHINGS

Lot	Maggots	pH of washings	Staph.	Strep.	Br. abortus		Typhoid
					No. 10	Page	
1	A, B and C	—	5	2.5	—	—	—
2	A	10.9	5	2.5	2.5	—	15
2A	A, 2nd washing	—	15	2.5	2.5	—	15
3	A, and D	9.6	2.5	2.5	2.5	15	30
4	A, 1st generation	10.0	—	—	—	5	—
5	B and C, 1st gen.	8.7	X	15	15	30	30
6	A, 1st gen.	8.5	—	—	2.5	5	—
7	B and C, 1st gen.	5.7	X	30	15	X	X
8	A, 1st gen.	7.9	30	2.5	—	—	—
9	A, B and C, 1st gen.	9.4	15	—	5	—	—
10	B and C, 1st gen.	7.1	—	—	30	30	—
11	B and C, 1st gen.	7.6	—	—	30	30	—
12	A, B, C and D, 1st gen.	6.3	X	30	—	—	—
13	A, B, C, D, mostly 2nd gen.	6.7	X	15	X	—	—
13A	A, B, C, D, mostly 2nd gen.	7.2	X	15	X	—	—
14	A, 3rd gen.	5.6	X	X	—	—	—
15	A, 3rd gen.	4.6	—	X	—	—	—
16	A, 3rd gen.	4.9	—	X	—	—	—
	Saline	5.8	X	X	X	—	—

Figures represent time in minutes at which transfers were sterile. X—growth from 30-min. tubes. Not examined further. A—*Calliphora erythrocephala* Mg. B—*Calliphora latifrons* Hough. C—*Cynomya cadaverina* Desv. D—*Calliphora vomitoria nigribarda* S.

TABLE II  
COMPARISON OF UNDILUTED AND DILUTED SAMPLES

Washings		Strep.	Br. abortus	
			No. 10	Page
Lot 1	—	2.5	—	—
Lot 1	1 : 100	60	—	—
Lot 2	—	2.5	2.5	—
Lot 2	1 : 100	30	30	—
Lot 2A	—	—	15	15
Lot 2A	1 : 10	—	30	30
Lot 2A	1 : 50	—	X	X
Lot 2A	1 : 100	—	X	X
Lot 4	—	—	5	5
Lot 4	1 : 10	—	30	30
Lot 4	1 : 20	—	30	30
Lot 4	1 : 50	—	XX	X
Lot 4	1 : 100	—	XX	X
Lot 6	—	—	5	5
Lot 6	1 : 10	—	30	30

Figures represent time in minutes at which transfers were sterile. X—growth from 30-min. tubes. XX—growth from 60-min. tubes.

(0.85% NaCl) by means of a double hydrogen electrode. Lots 2 to 11 and 14 to 16 had been kept in liquid form. The others had been dried and were resuspended in distilled water. The results are given in Table I. It will be noticed that the samples with higher pH values are, generally speaking, more bactericidal than those with a lower pH. Lot 5 appears to be the only exception. Lots 14, 15 and 16, prepared from the maggots of third generation flies, were the poorest of all, showing no bactericidal activity in 30 min. against the most easily killed organism, *Strep. mastitidis*. Lot 16 still showed growth after 60 min.

Although the various samples were not tested against the three organisms in all cases, there is sufficient information to show clearly the superiority of the earlier samples. It was noticed in the later samples that the color of the washings was not as dark and the suspensions did not appear as turbid.

#### BACTERICIDAL ACTIVITY OF DILUTED SAMPLES

A comparison was made of washings undiluted and diluted up to 1 : 100 with saline solution against *Strep. mastitidis* and two strains of *Br. abortus*. The results are shown in Table II.

The bactericidal activity rapidly disappeared on dilution at the time intervals employed. The suspensions were not tested after 60 min. and in some cases 30 min., as indicated in the table.

#### EFFECT OF SUSPENDING DRIED WASHINGS IN ALKALINE SOLUTIONS

In view of the results noted in connection with the pH reactions of the samples, dried washings from Lot 13 were suspended in a boric acid-potassium chloride-sodium hydroxide buffer solution and in Sørensen's glyccoll-sodium chloride-sodium hydroxide buffer solution, which were made after the method of Clark (2). Other suspensions were made in alkaline salt solution. Germicidal tests were made with these various solutions. There was a suggestion from the results of these tests that a high pH value is connected with bactericidal activity, although it may only be incidental, but on the whole the results were rather indefinite.

#### LOSS IN ACTIVITY OF DRIED AND LIQUID SAMPLES

Samples were tested after subjecting them to various conditions. These results are given in Table III. The advantage of storage in the dried rather than in the liquid state is evident.

TABLE III  
COMPARISON OF FRESH AND OLD SAMPLES

Lot	Age	Bactericidal action			
		Staph.	Strep.	Brucella	Typhoid
2	Fresh	5	2.5	—	—
	22 days (dried)	15	2.5	—	—
	80 days (dried)	30	2.5	—	—
2A	Fresh	15	2.5	2.5	15
	7 days (liquid)	30	30	15	X
3	Fresh	2.5	2.5	—	—
	22 days (dried)	15	2.5	—	—
5	Fresh	X	15	15	—
	7 days (dried)	X	15	30	—
	7 days (liquid)	—	30	30	—

Figures represent time in minutes at which transfers were sterile.  
30-min. tubes.

X—Growth from

The samples were poured on to a sheet of glass about 9 in. above a hot plate and dried in the draught of a fan. The material was scraped off and stored in vials at room temperature. The amount of dry material varied somewhat with different lots, the percentage yield of Lots 2, 3, 5, 9, 12, 13, and 13A being 1.8, 2.0, 1.3, 2.2, 1.4, 1.6, and 1.6, respectively. It should be remembered that 0.85% salt solution was used for washing the maggots and the salt is included in these weights. The dried material was brought back to the original volume with distilled water when required and was autoclaved at that time. There was no apparent relation between percentage of solids and bactericidal activity of the washings.

#### COMPARISON OF MAGGOTS, BEEF FROM WHICH THEY WERE TAKEN AND NON-MAGGOTY BEEF

Maggots from Lot 2, meat in which they had been raised, and a piece of the same meat not exposed to flies but kept under the same conditions,

TABLE IV  
BACTERICIDAL TEST OF MAGGOTS, MAGGOTY AND NON-  
MAGGOTY MEAT

Washings	Bacteria	Exposure in minutes			
		2.5	5	15	30
Maggots	Staph.	+	-	-	-
Maggoty meat	Staph.	+	+	-	-
Control meat	Staph.	+	+	+	+
Maggots	Strep.	-	-	-	-
Maggoty meat	Strep.	-	-	-	-
Control meat	Strep.	+	+	+	+

+ = growth in tubes of broth at 48 hr. after exposure indicated.

- = sterile after exposure indicated.

were washed with equal amounts of saline solution for a period of two hours. The washings were tested against *Staph. aureus*, and *Strep. mastitidis*. The results are shown in Table IV.

The meat in which the maggots had grown contained the bactericidal substance, whereas meat without maggots, kept under the same conditions, showed no trace of this substance.

#### COMPARISON OF BACTERICIDAL ACTIVITY OF MAGGOT WASHINGS AND A SUSPENSION OF GROUND MAGGOTS

Maggots (50 cc. in volume) from Lot 1 were washed, then ground with sand in 50 cc. saline. The mixture was filtered through coarse paper, autoclaved at 10 lb. for 20 min., and tested for sterility. The washings and the ground suspension were tested at the same time for bactericidal activity against *Staph. aureus*. Transfers were made at 2.5, 5, 15 and 30 min.; those from washings were sterile in 5 min. The suspension of ground maggots showed no bactericidal power. Growth was obtained from seedings made from the suspension the following morning.

#### ATTEMPT TO INCREASE BACTERICIDAL ACTIVITY AGAINST *Br. abortus* BY THE ADDITION OF THIS ORGANISM TO THE MEAT

An attempt was made to determine whether growth on specifically infected meat would increase the activity of maggot excretions against *Br. abortus*.

A portion of meat was heavily spread with the organism, while a second was left untouched. Both were seeded with eggs of the first generation of *C. erythrocephala*. Five days later, an equal volume of maggots was collected from each. These were washed in the usual manner, and both suspensions were tested against two strains of *Br. abortus*. There was no difference between the two lots (Lots 10 and 11 in Table I), both of which were low in bactericidal activity.

#### FILTRATION OF MAGGOT WASHINGS

Washings were filtered through paper and a Mandler candle. The material was difficult to filter, but a small amount of clear green fluid was obtained. Lot 2 filtrate did not kill streptococci in 30 min., while the suspension did so in 2.5 min. Lot 4 filtrate was tested against *Br. abortus*. The suspension tube was sterile at 5 min. and the filtrate tube at 15 min. Both showed growth with streptococci at 5 but not at 15 min. Lot 5 filtrate did not kill staphylococci or *Br. abortus* in 30 min. but killed streptococci in 15 min. The unfiltered material did not kill staphylococci in 30 min. but killed *Br. abortus* and streptococci in 15 min. Lot 8 filtrate did not kill staphylococci in 30 min. but killed streptococci in 5 min. Lot 8 suspension killed the former in 30 and the latter in 2.5 min.

Filtration, therefore, did not remove all the bactericidal activity but lowered it. It will be seen that the same holds true in regard to toxicity.

#### TOXICITY OF MAGGOT WASHINGS

Guinea pigs weighing from 350 to 400 gm. were given intraperitoneal injections of 1.0 cc. of various lots of washings and filtrates. All fresh lots caused a rapid fall in temperature, and in many cases death.

*Lot 1 fresh and after 5 days in refrigerator.* The temperatures of these guinea pigs are shown in Table V. The fresh material caused a rapid fall in temperature and death. In the second test there was some drop in temperature but recovery occurred rapidly.

TABLE V  
TEMPERATURES OF GUINEA PIGS INJECTED WITH FRESH WASHINGS AND THE SAME WASHINGS AFTER FIVE DAYS IN REFRIGERATOR

Sample	10:30 a.m.	11:30 a.m.	2:00 p.m.	4:00 p.m.	5:30 p.m.	9:00 a.m.
Fresh washings	102.4	96.2	94.0	94.0	94.0	Dead
Fresh washings	103.0	96.4	94.0	94.0	94.0	Dead
Old washings	103.0	102.2	101.8	101.4	102.2	103.0
Old washings	102.8	101.4	100.2	101.2	101.4	103.0

The reaction in these animals appeared to be very similar to that seen after injections of alcoholic precipitates of bacteria or fresh cow serum. There were marked signs of discomfort following the injection. The abdomen rapidly

became tense. In some cases there was a watery discharge from the eyes. The animals went off their feet in 3 or 4 hr., and death usually took place in 5 to 6 hr. Post-mortem examination showed a considerable amount of clear fluid in the abdominal cavity and intense inflammation of peritoneum. Cultures were usually sterile. In animals that died more slowly, a fibrinous exudate formed in the abdominal cavity, beginning around the edges of the liver. In a few instances, guinea pigs apparently recovered at the time but died a couple of weeks later.

*Lots 4 and 8 washings and filtrate.* One-cc. portions of filtered and unfiltered suspensions of each of these lots were injected intraperitoneally the day after preparation. The guinea pigs receiving unfiltered suspension showed a more rapid drop in temperature and were very ill for one to two days, whereas the animals receiving filtrate returned to normal in about one-half this time. Although none of the animals died, it was clear from their temperatures and general appearance that some of the toxic quality had been removed by filtration, just as the bactericidal activity is lowered.

#### COMPARISON OF A SAMPLE WHILE FRESH AND AFTER STORAGE IN DRY AND LIQUID FORM FOR THREE WEEKS

One portion of a toxic sample was kept in the refrigerator for three weeks. Another portion was dried and resuspended three weeks later, while a third

TABLE VI  
TEMPERATURES OF GUINEA PIGS

Sample	Time			
	3:00 p.m.	4:00 p.m.	5:00 p.m.	10:00 a.m.
Fresh washings	101.8	94.0	94.0	Dead
Liquid 3 weeks old	102.4	102.0	101.0	103.0
Dry 3 weeks old	102.0	98.2	94.0	102.8

was injected intraperitoneally into a guinea pig the day after preparation. The other two samples were subsequently injected in the same manner. Table VI gives the temperatures of three guinea pigs injected with 1.0 cc. of the suspensions.

There was a loss of toxicity in both samples that had been

held for three weeks, but it was more pronounced in that which had been stored in liquid form.

#### ANTIGENIC QUALITY OF MAGGOT WASHINGS

A rabbit was given five intravenous injections of a dried sample of washings over a period of 10 days. Blood was collected from the heart 10 days after the last injection, and sterile serum was prepared from it. This was employed in the complement fixation test and also tested for protective action in guinea pigs against a dried sample of washings.

*Complement fixation test.* The same lot of dried washings used for the production of the above serum was brought to its original concentration in water, autoclaved, and diluted 1 : 100, 1 : 1000 and 1 : 10,000 with saline solution. A sample was also heated at 56° C. for 30 min., in place of auto-

claving, and gave the same result as the autoclaved sample. Diluted antigen (1 cc.) was employed and undiluted serum was added to make dilutions of from 1 : 25 to 1 : 1000. Two units of complement were used. The hemolytic mix consisted of three units of a 1 : 6000 dilution of hemolysin in 0.25 cc. and 0.25 cc. of 2.5% sheep cells. The result of this test is shown in Table VII.

TABLE VII  
COMPLEMENT FIXATION TEST WITH MAGGOT WASHINGS AND ANTI-MAGGOT SERUM

Antigen dilution	Serum	Serum dilution						Antigen alone	Serum alone		
		1 : 25	1 : 50	1 : 100	1 : 250	1 : 500	1 : 1000		1 : 25	1 : 50	1 : 100
1 : 100	Anti-maggot	4	4	4	4	4	4	0	0	0	0
1 : 1000	Anti-maggot	4	4	4	4	0	0	0	-	-	-
1 : 10,000	Anti-maggot	0	0	0	0	0	0	0	-	-	-
1 : 100	Normal	0	0	0	0	0	0	0	0	0	0

4 = complete fixation; 0 = complete hemolysis.

The suspension when diluted 1 : 100 and 1 : 1000 made good antigen with this anti-maggot serum. No fixation of complement occurred with the 1 : 10,000 dilution. The experiment also showed that the washings were capable of producing a good complement-binding serum. Normal rabbit serum produced no fixation in combination with these antigen dilutions.

#### BACTERICIDAL TESTS *in Vivo*

Experiments were carried out with the object of protecting guinea pigs against infection by intraperitoneal injections of maggot washings, which had been sufficiently diluted to avoid the toxic action of the substance. *Br. abortus* was used for the infecting agent because in previous work this organism had proved to be very consistent in infecting guinea pigs. The work was carried out in a manner similar to that employed in the anti-abortion serum experiments of the senior author (4).

The guinea pigs were infected in some experiments by injecting a light suspension of *Br. abortus* intraperitoneally, in others by placing a drop of the suspension in the eye. Some of the guinea pigs in each group were then given daily injections of dilutions of 1 : 5 to 1 : 50 of maggot washings. Different dilutions were used in different experiments and the time of commencing treatment with them was varied.

There was a delay in agglutinin production in the treated animals, but they were all found to be infected on post-mortem examination.

#### ACTION OF MAGGOT WASHINGS ON GUINEA PIG ERYTHROCYTES *in Vitro*

Four lots of dried washings were brought to their original concentration and autoclaved in the usual manner. These, and six additional fluid samples, were added to portions of a 1% suspension of washed guinea pig red cells. Cell suspension (1 cc.) was placed in small tubes and to these 0.01, 0.1 and

1.0 cc. of each sample were added. The tubes were then incubated for one hour in the water bath at 37° C. The results are shown in Table VIII.

TABLE VIII  
ACTION OF MAGGOT WASHINGS ON GUINEA PIG RED CELLS *in vitro*

Lot	State	0.01 cc.	0.1 cc.	1.0 cc.
5	Fluid	No change	Partial hemolysis	Partial hemolysis
6	Fluid	Hemolysis	Hemolysis	Hemolysis
7	Fluid	Agglutination	Agglutination	Hemolysis
8	Fluid	Hemolysis	Hemolysis	Hemolysis
10	Fluid	Agglutination	Agglutination	Hemolysis
11	Fluid	Agglutination	Hemolysis	Hemolysis
2	Dried	Agglutination	Hemolysis	Hemolysis
3	Dried	Hemolysis	Hemolysis	Hemolysis
5	Dried	Agglutination	Hemolysis	Hemolysis
9	Dried	Agglutination	Partial hemolysis	Hemolysis

It was rather difficult to determine what had taken place in the 0.1 and 1.0 cc. tubes, on account of the dark color of the washings. A heavy precipitation was noted in both tubes when hemolysis had occurred. A rather clear, green supernatant fluid remained. The sediment did not contain blood cells. Evidently the cells were hemolyzed and the hemoglobin was quickly precipitated. The smallest dose usually agglutinated the red cells without any hemolysis, but in two instances complete hemolysis occurred in these tubes. It will be noticed that Lot 5 fluid was not as active as the dried sample of this lot. Both were examined 18 days after preparation.

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## ANTI-BRUCELLA SERUM: GUINEA PIG PROTECTION EXPERIMENTS<sup>1</sup>

BY RONALD GWATKIN<sup>2</sup>

### Abstract

Guinea pigs were protected against infection with *Brucella abortus* by intraperitoneal injections of fresh, unpreserved, anti-*abortus* rabbit serum. In the first two experiments the serum-treated and control animals were exposed to infection by contact with infected guinea pigs, while in the third experiment they were exposed by instillation of the infecting organism into the eye. In the first experiment 60% of the controls and none of the treated animals were infected. In the second, 80% of the controls and 20% of the treated animals were infected. In the third, all of the treated animals were protected, while all of the controls became infected. The former method, although less certain than the latter, is worthy of consideration in experiments of this type, as it permits infection to occur in a natural manner.

### Introduction

In previous papers the writer (2-4) reported that repeated injections of the serum of immunized guinea pigs and rabbits protected normal guinea pigs against infection with *Brucella abortus* by mouth and eye. Serum did not prevent the development of infection in animals infected prior to receiving injections of serum. Cow and horse serum were much less satisfactory in prophylactic experiments with guinea pigs.

The work of Beach (1) confirmed our results with cow serum in guinea pigs. He found that the protective power of such serum for these animals was not very high.

Mitchell, Humphreys and Walker (5) found that anti-*Brucella* horse serum conferred a degree of protection when administered to guinea pigs previous to a challenge inoculation of *Br. abortus*, but if administered after infection was established it did not confer any protection.

In view of our results with anti-*abortus* rabbit serum in the protection of guinea pigs against exposure to *Br. abortus* by mouth and eye, it was decided to study the effects of injecting such serum into animals exposed to infection by contact with artificially infected guinea pigs. Such an exposure would be less severe than the artificial methods employed in the previous experiments, but might also be expected to be less certain.

### EXPERIMENT 1. PREVENTION OF NATURAL INFECTION

*Preparation of serum.* Ten large rabbits were given four weekly injections of an old strain of *Br. abortus*. Growth from the flat surface of beef agar in three 16-ounce medicine bottles was collected (after three days' incubation at 37° C.) in 25 cc. of salt solution, and 0.5 cc. was injected intraperitoneally the first time, followed by injections of 2.0 cc. Thirty-five days later these rabbits were given an intraperitoneal injection of 0.5 cc. of a light suspension

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of a recently isolated strain of *Br. abortus* (No. 2 McFarland nephelometer). A week later the growth from two liver agar slants was collected in 20 cc. of salt solution, and the rabbits were given intraperitoneal injections of 2 cc. of this suspension. These injections were continued throughout the experiments.

About 20 cc. of blood was drawn from the heart of each rabbit once a week and the blood was allowed to clot in sterile vials. The withdrawal of two lots of 10 cc. with a short interval between operations was found to be safer than withdrawing 20 cc. at one time. The serum was collected aseptically and stored without preservative in the refrigerator until used. It was usually injected the same week in which it was collected. In this way a much greater quantity of serum was obtained than would have been possible if the rabbits had been sacrificed for serum collection, and fresh unpreserved serum was available for each injection. Guinea pigs would have been more satisfactory as sources of serum, as serum injections could have been given to the experimental animals after longer intervals. Guinea pigs are much less resistant to *Br. abortus* than rabbits, and the mortality during serum production is high. Agglutination tests were carried out with blood obtained each week prior to the injections of *Br. abortus*. After the first rise to 1 : 10,000 in many of the rabbits, the titre of all varied between 1 : 1000 and 1 : 2500 with an occasional rise in some to 1 : 5000.

*Experimental guinea pigs.* Thirty-two guinea pigs, one group of seven and five groups of five, were used in this experiment. Seven were exposed to infection by eye with one drop of a suspension of *Br. abortus*. Seven days later the untreated group and the four serum-treated groups were placed in the same pen. Intraperitoneal injections of 2 cc. of serum were given to the treated groups at various intervals. The groups were as follows:

- Group 1. Infected; 3 males and 4 females.
- Group 2. Untreated; 3 males and 2 females.
- Group 3. Serum 3 times a week; 3 males and 2 females.
- Group 4. Serum twice a week; 3 males and 2 females.
- Group 5. Serum once a week; 2 males and 3 females.
- Group 6. Serum every 2 weeks; 2 males and 3 females.

Three of the last group, as might have been expected, died from anaphylactic shock, as did also one of Group 5. The two groups were then combined and given serum once a week.

The strain of *Br. abortus* employed to infect the guinea pigs of Group 1 was not very virulent, although recently isolated, because at 29 days only one showed agglutinins. The controls (Group 2) were negative at this time. Group 1 was then injected intraperitoneally, and two animals died from peritonitis the following day. A week later the five survivors of this group were positive by the agglutination test. Failure of the first exposure to produce infection prolonged the experiment and necessitated giving more serum injections than would otherwise have been required. The groups

were bled a week later, at which time the sera of the animals in Group 1 had agglutinin titres of 1 : 1000 or more, while the controls were still negative. At the fifth weekly bleeding one control was found to be positive, a second was found positive at the sixth week, and a third at the seventh week. The other two remained negative.

On April 13, three months from the beginning of the experiment, the last injection of serum was given and the groups were placed in separate cages. For some undetermined reason a few of the serum animals died after removal from the pen to cages. There was no intercurrent infection and we were fortunately able to determine the absence of any evidence of *Brucella* infection. Details of these animals are given later. The serum guinea pigs were all positive at this time but subsequently ceased to react, while the control group and the infected group remained unchanged, *i.e.*, three positive and two negative in the control and all positive in the infected group. They were killed on May 12, blood was collected for the agglutination test, and cultures were made. The results follow.

*Group 1 (Infection only).* Five guinea pigs. All were emaciated, two lost the use of their hind limbs, spleens of all were enlarged and nodular, lesions in testes of the two males. Agglutinin titres ranged from 1 : 250 to 1 : 1000. Cultures from four yielded *Br. abortus*.

*Group 2 (Controls).* Five guinea pigs. Three were positive by the agglutination test and two were negative. The spleens of the former were enlarged and *Br. abortus* was recovered from them on culture. The latter showed no lesions and cultures remained sterile.

*Group 3 (Serum three times a week).* Five guinea pigs. Two males died about 10 days after being placed in cages. In both cases the gastric mucous membrane was covered with punctate haemorrhages, which could also be observed under the serous coat. No other changes except emaciation were noted. The spleens of these animals were normal in appearance, cultures were negative and only a trace of agglutination at 1 : 25 remained. Their serum titres had previously been 1 : 100 and over, owing to injections of serum. The other three animals were negative at the time they were killed, spleens were normal and cultures remained sterile.

*Group 4 (Serum twice a week).* Five guinea pigs. One male died about the same time as the two in Group 3. It showed the same condition of the gastric mucous membrane. The serum still had a slight agglutinative power shortly before death, due to the injections of positive serum, but post-mortem findings and cultures showed no evidence of infection. The other four animals were negative when they were killed, spleens were normal and cultures negative.

*Group 5 (Serum once a week).* One of this group of five guinea pigs died early in the experiment from anaphylactic shock. One of the remaining four died May 2 and the same gastric lesions were observed as in those that died of Groups 3 and 4. No agglutinins were demonstrated in the blood,

the spleen was normal in appearance and cultures were sterile. One male and two females survived. They were negative by the agglutination test, spleens were normal and cultures negative. These females each had one young guinea pig several weeks old at the conclusion of the experiment. The young animals were also shown to be free from infection by the agglutination test, cultures, and absence of lesions.

*Group 6 (Originally serum every two weeks but changed to once a week).* Three of the five animals in this group died of anaphylactic shock after the second injection of serum, and the survivors were then given serum once a week as in Group 5. These animals showed no agglutinins at the time they were killed, spleens were normal in appearance and cultures were negative.

### *Results of Experiment 1*

In view of the fact that intra-abdominal injections of serum were given to Groups 3 to 6 from one to three times a week, no particular importance could be attached to the birth of young in this experiment. In all the serum groups some females carried their young to full term, in spite of the injections, but the young animals, with the exception of those in Group 5, died after a few days from lack of nourishment as the females had little or no milk. There was no difference in protection in the groups receiving one or three injections each week. Three of the five control guinea pigs became infected (60%). Four of the serum animals died at the beginning from anaphylactic shock. None of the remaining 16 showed any evidence of infection at the conclusion of the experiment. There would have been a more clear-cut picture if all the controls had become infected, but under natural conditions of infection this could hardly be expected. None of the serum-treated animals became infected and it is quite plain that they were completely protected under conditions that caused the infection of 60% of the control group.

## EXPERIMENT 2. PREVENTION OF NATURAL INFECTION

In the first experiment there did not appear to be a sufficiently large group of infected animals to cause a rapid spread of infection to the controls. In the present experiment this was made the largest group. One group of serum-treated animals and a control group of the same size were used. Injections of rabbit serum were given twice a week to avoid the danger of anaphylactic reactions.

*Preparation of serum.* Serum from the rabbits used in Experiment 1 was used for protective injections. Weekly bleedings and injections of *Br. abortus* were continued as formerly, and more rabbits were immunized in the same manner, but, as sufficient serum was available from the first lot, these were not bled until the thirteenth injection on May 26.

*Experimental guinea pigs.* Thirty-five guinea pigs were used. Group 1 consisted of 15 animals, which were infected by intraperitoneal injection. Group 2 consisted of 10 untreated controls, and Group 3 of the remaining 10, which were injected with serum. All were placed in the same pen on April 14,

at which time Group 1 was infected and Group 3 received the first injection of serum. Groups 1 and 2 were bled at weekly intervals starting the second and third weeks after infection, and were killed June 16, nine weeks after the beginning of the experiment. Particulars of these groups are given under their respective headings.

*Group 1 (Infection only).* Fifteen guinea pigs infected by intraperitoneal injection of a suspension of *Br. abortus* on April 14. One female died of peritonitis seven days after injection. The organism was recovered in pure culture. All the others appeared ill but recovered, although a few continued to drag their hind legs. The 14 survivors were bled two weeks after injection and the agglutination test was set up to 1 : 200, at which dilution all sera showed complete agglutination. There were nine males and five females. These animals had gained only an average of 55 gm. each when they were killed on June 16. The spleens of all were considerably enlarged, the testes of the males were atrophied but were not abscessed. None of the females were pregnant. *Br. abortus* was recovered from 13 of the 14. Four had an agglutinin titre of 1 : 500 and ten of 1 : 1000 or over.

*Group 2 (Controls).* Ten untreated guinea pigs, seven of which were males. They were weighed, properly identified and placed in the same pen as Group 1. They were bled on May 5, three weeks after the beginning of the experiment, and at weekly intervals thereafter. The first test showed two males and one female were infected. The number gradually increased, as shown in Table I, and on June 16, when they were killed, eight were infected.

TABLE I  
AGGLUTINATION TESTS ON GROUP 2 (CONTROLS)

No.	Sex	May				June		
		5	12	19	26	2	9	16
1	M	—	—	—	—	—	—	—
2	M	1 : 25	1 : 25	1 : 100	1 : 100	1 : 100	1 : 250	1 : 500
3	F	1 : 25	1 : 100	1 : 100	1 : 100	1 : 100	1 : 250	1 : 100
4	M	1 : 100	1 : 100	1 : 250	1 : 500	1 : 1000	1 : 1000	1 : 1000
5	M	—	—	—	—	1 : 50	1 : 100	1 : 250
6	F	—	—	1 : 25	1 : 25	1 : 50	1 : 100	1 : 250
7	F	—	1 : 50	1 : 100	1 : 100	1 : 250	1 : 100	1 : 100
8	M	—	—	—	—	—	—	—
9	M	—	—	—	—	—	—	1 : 100
10	M	—	—	—	—	1 : 50	1 : 100	1 : 100

Nos. 1 and 8 (Table I) were found to be normal on post-mortem examination, and cultures were negative. The spleens of all the others except No. 9 were enlarged, and *Br. abortus* was recovered from all except No. 3, cultures of which were overgrown with mold. No. 6 was pregnant and *Br. abortus* was recovered from the foetal membranes in addition to the spleen. This group had made an average gain of 131 gm. at this time.

**Group 3 (Serum twice a week).** Ten guinea pigs, of which six were males. They were given an intraperitoneal injection of 2 cc. of serum on April 14, and twice a week thereafter, until a total of 19 injections had been given; the last was given on June 16, at which time eight of the controls were positive to the agglutination test. They and the animals of Group 1 were killed, while those of Group 3 were placed in clean quarters after receiving the last injection of serum. On this date the animals of this group showed an average gain of 212 gm. each. Agglutination tests were not carried on during the serum injection period but were begun on the day of the last injection, when the serum titres ranged from 1 : 50 to 1 : 100. Results of these tests are shown in Table II.

TABLE II  
AGGLUTINATION TESTS ON GROUP 3  
(SERUM-TREATED GUINEA PIGS)

No.	Sex	June			July
		16	23	30	6
1	M	1 : 100	1 : 50	1 : 25	—
2	M	1 : 100	1 : 50	—	—
3	F	1 : 50	1 : 25	1 : 25	—
4	M	1 : 100	1 : 25	—	—
5	M	1 : 50	1 : 200	1 : 100	1 : 100
6	M	1 : 50	1 : 25	1 : 25	—
7	M	1 : 50	1 : 50	—	—
8	F	1 : 100	1 : 100	1 : 100	1 : 100
9	F	1 : 50	1 : 25	—	—
10	M	1 : 50	1 : 25	—	—

The guinea pigs of Group 3 were killed on July 7. Post-mortem examination showed all to be normal except Nos. 5 and 8. The former had an enlarged spleen and in the latter the spleen was large, nodular and somewhat haemorrhagic. This animal showed an early pregnancy. The other two females were also pregnant, No. 9 being practically at full term. Cultures from all were negative but the presence of lesions and persistence of agglutinins

in Nos. 5 and 8 clearly indicate that these two animals were infected.

**Results of Experiment 2.** The strain of *Br. abortus* used in this experiment was evidently quite satisfactory, judged by the appearance of lesions in Groups 1 and 2.

Groups 1 and 2 were killed on June 16, at which time serum injections for Group 3 were stopped, and the animals were placed in clean quarters, where they remained until killed on July 7. On June 16 the average gain in weight of the original infected group was 55 gm., that of the control group 131 gm., and that of the serum-treated group 212 gm.

All the animals in Group 1 were infected. Eight of the 10 untreated controls became infected (80%) while only two of the serum-treated group showed evidence of infection at the conclusion of this experiment (20%).

### EXPERIMENT 3. PREVENTION OF INFECTION BY EYE

While Experiment 2 was in progress a small group was treated along the lines reported in earlier papers, to which reference has been made. This was done in order to have a direct comparison between the severity of natural exposure and artificial exposure by eye, with the same serum as used in Experiments 1 and 2 and the same culture as used in Experiment 2.

**Experimental guinea pigs.** Two groups of three guinea pigs were employed. Each animal in Group 1 was given 2 cc. of serum intraperitoneally on May 9. Group 2 was left untreated. The following day both groups were exposed to infection by eye with one drop of a suspension of the same strain of *Br. abortus* as used in the previous experiment. The suspension was of such a density that when diluted with three parts of saline it was equal to Tube No. 1 of the McFarland nephelometer. Six injections of 2 cc. of serum were given to Group 1 over a period of three weeks and both groups were bled for the agglutination test and weighed once a week. Agglutination results are given in Table III.

TABLE III  
AGGLUTINATION RESULTS IN EXPERIMENT 3

Group	No.	Sex	May				June				July
			9	16	23	30	6	13	20	27	4
I	1	F	—	1 : 100	1 : 50	1 : 50	1 : 25	1 : 25	—	—	—
	2	M	—	1 : 100	1 : 100	1 : 50	1 : 50	1 : 25	—	—	—
	3	F	—	1 : 100	1 : 100	1 : 100	1 : 100	1 : 100	1 : 100	1 : 50	—
II	1	F	—	—	1 : 25	1 : 100	1 : 100	1 : 100	1 : 100	1 : 100	1 : 100
	2	F	—	—	1 : 25	1 : 100	1 : 100	1 : 100	1 : 100	1 : 100	1 : 100
	3	F	—	—	1 : 50	1 : 100	1 : 100	1 : 100	1 : 100	1 : 100	1 : 100

All were negative at the beginning of the experiment and prior to the injection of serum. The following week the serum animals of Group 1, as would be expected, were positive, while the untreated ones had not yet developed agglutinins, because of infection. These were first observed two weeks after the beginning of the experiment.

The serum-treated group lost 60 gm. the first week as compared with 20 gm. in the control group. When taken from the pens and placed in wire-bottomed cages guinea pigs always show a temporary loss in weight, such as occurred in Group 2. In Group 1 the greater loss in weight can only be ascribed to the serum injections. Intraperitoneal injections of almost any substance will cause similar losses in weight. Both groups showed a loss in weight at the conclusion of the experiment, the loss in Group 1 still being the greater, namely, 42 gm. against 13 gm. in Group 2.

On May 26 the last injection of serum was given to Group 1. Weekly bleedings and weighings were continued until July 4, when all the serum-treated animals were negative and both groups were killed. There were no gross lesions in either group but cultures from Group 2 were positive while those from the serum-treated group were negative.

**Results of Experiment 3.** The serum-treated group was protected against eye infection by six injections of serum. Agglutinins had disappeared at the conclusion of the experiment, and cultures were negative. The control animals showed agglutinins, and cultures yielded *Br. abortus* from all spleens

but no gross lesions were observed. Both groups lost weight, the loss being greater in the serum-treated group.

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# WOOL GROWTH AND QUALITY AS AFFECTED BY CERTAIN NUTRITIONAL AND CLIMATIC FACTORS\*

## III. RESULTS AND CONCLUSIONS<sup>1</sup>

By J. E. BOWSTEAD<sup>2</sup> AND P. LAROSE<sup>3</sup>

The purpose of this investigation and the experimental treatment of the sheep were described in Part I (1) of the series, and the laboratory methods of testing the wool were described in Part II (2). This paper presents the results obtained by these methods, and completes the series.

### Results

The differences in the performance of animals between any two experimental groups are largely dependent upon the differences in the conditions imposed.

As stated in Part I, the experimental periods in the later trials were lengthened to ensure greater significance to the results. Temperature differences varied from 23.8 to 52.2° F., owing to differences in the average outdoor winter temperatures and the temperatures that were maintained in the heated pens.

Humidity differences between Lots I and II were necessarily dependent upon the differences between outdoor and indoor temperatures, since cold winter air was brought into these two lots and was raised by an average of from 23 to 52° F. In Lot I a humidifier raised the relative humidity to the outdoor relative humidity. In Lot II, with no humidifier, the relative humidity varied from 7.4% to 31.5% less than in Lot I, which variation was due in part to yearly differences in average outdoor and indoor temperatures and to improved ventilation of pens during the last three years.

The differences between the conditions imposed on other comparable groups of ewes varied according to kind and amounts of feeds and were more readily controlled by those conducting the trials.

The differences between comparable groups were greatest in the 4th trial.

A brief summary of the differences imposed on the comparable groups is given in Table I.

### *Effect of Humidity*

The effect of increasing humidity in heated pens did not cause any significant results. While the data for the five years show an average decrease in fibre length of  $6.2 \pm 3.5\%$ , the greater part of this average decrease was due to the results obtained during the first two trials when humidity differences were less than in later trials. In the fourth year, when humidity differences were the greatest, the decrease in fibre length was only  $1.7 \pm 10.4\%$ , which reduced the significance of the five-year average result.

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\*Presented in a series of three papers.

The differences in fibre diameters and tensile strength show little or no effect of humidity differences. The examination of the fleeces of the two groups at Weston also showed little or no difference in fineness or strength.

TABLE I  
DIFFERENCES IN CONDITIONS IMPOSED

Trial	Av. temp. diff. between Lots I and III, °F.	Av. relative humidity diff. between Lots I and II, %	Plane of nutr'n daily TDN* fed, Lots			Per cent more protein fed to Lot VIII than to Lot VI	Amt. more mineral fed Lot IX than Lot VI daily, lb.
			V lb.	VI lb.	VII lb.		
1st	41.8	7.4	1.69	2.04	2.06	43	.012
2nd	23.8	8.2	1.95	2.11	2.30	31	.010
3rd	37.5	15.8	1.79	1.86	2.09	44	.012
4th	52.2	31.5	.91	1.41	1.89	90	.016
5th	42.0	25.4	1.19	1.56	1.65	39	.010

\*Total digestive nutrients.

While the weighted average of the grease data is not significant, it may be noted that in the 4th trial, in which the largest humidity differences were obtained, the ewes in the high humidity pen produced fleeces considerably lower in percentage of grease than those produced by the ewes in the drier pen. The ewes in the high humidity pen, however, produced fleeces with a slightly greater suint content in every trial.

While the results indicate that the higher humidity condition may slightly affect certain characters of wool growth and quality, there is insufficient evidence to believe that under usual winter management practices humidity differences could be great enough to materially affect the growth of wool or its quality.

### *Effect of Temperature*

In four out of five trials the wool produced by the ewes in the low temperature group was longer than wool produced by the ewes in the heated pen. The difference in average length of wool between the two groups was especially large in the 1st trial, when the average temperature difference was fairly large, and moderately so in the 4th trial, when the temperature difference was greatest; but there was sufficient variation in the data to reduce the significance of the results below that usually accepted for such trials.

There was also a tendency for the higher temperature to cause a reduction in fibre diameter and tensile strength. These changes, while not significant, are not contrary to the frequent observation that any retardation in wool growth is accompanied by increased fineness and fibre weakness. Differences in fineness and strength were not noted when the fleeces of the two groups were examined at Weston, where the examination was not confined to that small portion of the fibre that grew during the experimental period.

In four of the five trials the fleeces produced under high temperature conditions averaged from 3.1 to 5.4% less in clean wool. The difference in percentage of grease between the fleeces of the two groups varied both ways in the different trials, so that the difference in percentage of clean wool may have been due to dirt and suint.

From these results it could be concluded that when fair shelters and bedding are provided, there is no advantage in keeping sheep in heated pens during the winter time at temperatures such as existed during these five trials.

### *Effect of Early Shearing*

During the first four years, the ewes that were used in both lots had been sheared at the end of the previous May or beginning of June, and therefore carried about six months' wool growth when fall samples were taken. Only samples of fleece were taken for laboratory analysis at the end of February or beginning of March from those in the "early sheared" group. Similar samples were taken from the "late sheared" group at the usual shearing time, when the entire fleeces were removed from both lots. The data are, therefore, based on early and late samples removed from the ewes, the early samples representing about nine months' fleece growth and the samples taken at the usual time from the late sheared group representing 12 months' growth.

This accounts for the fact that, for the first four years, the late sheared group have fleeces from 25 to 40% longer than the early sheared fleeces. When both groups of fleeces represented 12 months' growth, the early sheared fleeces were slightly longer, but the difference was not significant.

The significant differences between the two groups were in the diameter and tensile strength measurements. In four of the five trials the average fibre diameters and tensile strength were greater for the early sheared fleeces. There may be two possible explanations for these results. The natural tendency for some or all sheep to "shed" their wool in the early spring months is characterized by a thinning and weakening of the fibres at the growing point. When sheep are sheared late this thin and weak portion is easily detected on grading. On the other hand, when ewes are sheared early, before the "shedding" process has commenced, the thinner and weaker portion becomes the tip of the following year's fleece and is probably not as easily detected under the usual grading methods. The second explanation for the effect of early shearing may be that when sheep are sheared early they do not undergo the discomfort of carrying a heavy fleece during the warmer days of spring, which may alter the rate of growth and size of the wool fibre as it is formed in the follicle.

As previously mentioned, the data obtained during the first four years were obtained from samples removed at the start of the trials and at the different times of shearing. The entire fleeces of both groups were removed at the usual or "late" shearing time. Therefore, fleece weight data for the first four years cannot be compared. In the 5th trial the difference of 2.2 lb. in favor of the late sheared group can be shown to be chiefly due to inherited individual differences in wool production. In 1936, two years later, six of

the ewes that had been in the late sheared group in the 5th trial gave fleeces that averaged 1.1 lb. heavier than the fleeces from six of the ewes that had been in the early sheared group of the same trial, although all were exposed to identical conditions.

In every trial the early sheared fleece samples had less shrink, or in other words a greater percentage of clean wool, than the late sheared fleece samples. This would indicate that there may be an increased secretion of grease and suint between the time of "early" and "late" shearing or approximately between March 1 and June 1.

The results of the inspection of the fleeces at Weston in 1933 supported the results of the laboratory tests on the fleeces, in that the coarser fleeces were produced by sheep sheared about March 1. However, in 1934 no differences were noted. According to Mr. W. H. J. Tisdale of the Canadian Co-operative Wool Growers Ltd., earlier shorn fleeces examined at the warehouse were superior to later shorn fleeces.

Sufficient data have been presented to show that earlier shearing can be recommended as a means of strengthening the fleece and reducing the shrinkage. . Delay in shearing may reduce the value of the fleece.

#### *Plane of Nutrition*

The three groups of ewes in this study, at low, medium and high planes of nutrition, were supposed to be kept on sub-maintenance, maintenance and fattening rations respectively. Owing to individual differences and to differences in amount of feed eaten (because of group feeding), there was considerable variation in the gains made by the ewes in any one group.

Prior to the first four trials the ewes had been on pasture during the entire pasture season. The 5th trial began in June after the ewes had been sheared at the end of the 4th trial. All groups, therefore, were transferred from the 4th trial into the 5th trial without being allowed the usual pasture period. Almost all the ewes became unthrifty in all lots, failed to eat their respective feed allowances and lost considerable weight. The results of the 5th trial cannot, therefore, be regarded as significant.

Significant differences in wool growth and quality were found between the fleeces produced by ewes on the low and medium plane rations, and also between the fleeces produced by the ewes on the low and high plane rations. Less significant results were secured when the fleeces of the ewes on the medium and high plane diets were compared. Such results are in accord with those of numerous workers who conclude that wool growth and quality could be materially affected only when normal thrift and health were greatly altered.

The ewes fed the medium and high plane rations produced fleeces that were significantly longer in staple and heavier both on raw and clean wool bases than the fleeces produced by the ewes on the low plane diet. There was a tendency also for the fleeces of the ewes on the higher plane diets to be greater in diameter and strength with a greater percentage of grease than the fleeces produced in the low plane lot. This heavier grease percentage may have been the cause of the greater shrink.

The results of the examination of fleeces at Weston in 1932, 1933, and 1934 corresponded favorably with the laboratory results. In general, the fleeces of the low plane group were consistently weaker with less lustre and lower yields.

It may be stated, therefore, that when ewes are fed inadequate rations and as a consequence become thin and unthrifty, wool growth and quality are adversely affected. The data would also tend to show that a normal fleece growth was secured by maintaining the ewes in a fair condition of flesh since still heavier feeding, as in Lot VII, did not stimulate a significantly greater production of wool.

### *Effect of Protein*

Casein was chosen as the protein supplement because it contained little of other nutrient factors that are of physiologic significance, and because of its low cystine content, which might have an influence on wool growth and quality.

The amount of casein fed varied. As the casein was fed in the concentrate mixture, the difference in the protein intake and the difference in the nutritive

ratio of the low and high protein rations depended on both the amount of concentrate mixture fed and amount of roughage consumed.

Based on roughage and concentrate consumption, the nutritive ratio of the two rations and length of experimental periods for the two groups were as shown in Table II.

TABLE II

Trial	Lot VI, low protein	Lot VII, high protein	Length of experimen- tal period, days
1st	1 : 10.0	1 : 7.0	98
2nd	1 : 9.8	1 : 7.4	143
3rd	1 : 9.2	1 : 6.7	154
4th	1 : 8.6	1 : 4.2	202
5th	1 : 9.1	1 : 6.5	370

The first three trials are somewhat similar for differences in the nutritive ratio of the rations, but in the 4th trial the high protein group received 90% more protein because of a smaller hay and larger concentrate allowance.

Even in those trials where the differences in the amount of protein were greatest, or where the experimental periods were longest, the feeding of increased amounts of casein (technical) did not affect the growth or quality of wool. The individual trends for the wool characters studied were very variable throughout the five trials. The fleeces of the two groups also showed little or no difference when examined at Weston.

These results are not in agreement with those of some earlier investigators, but the results of later experiments agree with or help to explain those obtained in these trials. There may be several reasons why the negative results were secured:

- (i) The ewes were mature and therefore required less protein.
- (ii) Casein is an unbalanced protein, being low in cystine, and probably could not be utilized efficiently.
- (iii) Casein did not contain other physiologically active substances, as contained in some protein supplements used by other investigators.

- (iv) Sufficient protein may have been fed the ewes in the low protein lot to meet their requirements.

### *Effect of Minerals*

The percentages of the mineral supplements in the grain mixtures were as follows:

- 1st trial—2% calcium carbonate, 1% sodium sulphate.
- 2nd trial—2% calcium carbonate, 1% sodium sulphate.
- 3rd trial—2% calcium lactate, 1% sodium sulphate.
- 4th trial—1½% calcium lactate, 1% sodium sulphate, 1½% mono-sodium phosphate.
- 5th trial—1½% calcium lactate, 1% sodium sulphate, 1½% mono-sodium phosphate.

The amount of mineral supplement consumed by the high mineral group depended on the amount of grain fed. The greatest amount consumed was in the 4th trial, when the feeding period was fairly long, grain consumption fairly high, and the percentage mineral supplement 4%.

Regardless of the length of trial, amount of supplement or kind of minerals fed, no significant effect on wool growth and quality was noted. It would appear that the basal ration of oats and Prairie hay, oat hay, or Western Rye, provided sufficient minerals for normal wool growth and quality. Had the ewes been immature or had the soils of the districts in which the feeds were grown been abnormally low in these minerals, the results might not have been the same.

### **Conclusions**

The maintenance of sheep in a heated pen at the same relative humidity as normal winter outdoor humidity did not increase wool growth or improve quality above that produced by ewes maintained in a heated pen with a considerably lower relative humidity. This indicates that, under the usual methods of management and climatic conditions, there would be no advantage in increasing the relative humidity of heated pens in areas where low humidity conditions occur naturally.

The maintenance of sheep in heated pens during the winter months, when periods of extremely low temperatures often occurred, had little effect on wool growth and quality. This indicates that there is no advantage in keeping sheep in heated pens if adequate shelter is supplied.

Early sheared fleeces were slightly coarser and stronger than late sheared fleeces. This suggests that sheep should be sheared as early as weather conditions permit, and where suitable shelters can be provided in case of inclement weather.

The feeding of sub-maintenance rations to pregnant ewes during the winter season seriously reduced the wool growth and quality. Maintenance of the ewes in a fair condition of flesh resulted in the production of fleeces that could be regarded as normal. Heavy feeding or maintenance of ewes in a high condition of flesh slightly increased the weight of fleece (five-year average, 0.3 lb. clean wool). To obtain normal lamb crops and fleeces normal in both

weight and quality, it appears most profitable to maintain sheep in a fair condition of flesh.

Mature ewes, when fed larger quantities of a protein supplement, did not produce fleeces that were heavier or of better quality than those produced by ewes on a basal ration of non-leguminous hays and oats. The results indicate that the purchase of high-priced protein supplements is not advisable when a fair basal ration is fed.

Mature ewes, when fed non-leguminous roughages and oats plus calcium, phosphorus and sulphur-containing salts, did not produce fleeces any heavier or of better quality than ewes not fed these mineral salts. Except in areas where the soil is especially deficient in these mineral elements, there does not appear to be any practical value in feeding these mineral supplements when the ewes can be maintained in a fair condition of flesh on a non-leguminous ration. (It is essential that all sheep be fed common salt and iodine in proper quantities.)

### Appendix

#### MATHEMATICAL METHODS USED

As indicated in Part II, wool samples were taken from each sheep at the beginning and end of the experimental periods, and sufficient measurements were made on every sample for each character to secure a fairly accurate average measurement.

In this manner average figures for each animal were available for further study. For example, in the 4th trial the figures for fibre length were as follows:

Ewe No.	Nov. sample, cm.	June sample, cm.	Difference, cm.	Difference, %	$d^2$
<i>Lot V</i>					
374	5.0	9.0	4.0	80	289
379	5.0	10.2	5.2	104	49
384	4.7	8.9	4.2	89.4	57.8
385	5.1	8.9	3.8	74.5	506.2
671	4.6	9.8	5.2	113	256
675	4.3	9.5	5.2	120.9	571.2
689	6.3	12.0	5.7	90.5	42.2
700	4.6	9.7	5.1	110.9	193.2
Total	39.6	78.0	38.4		1964.6
Average	5.0	9.8	4.8	97 Table III	
<i>Lot VI</i>					
361	5.0	9.5	4.5	90	1142.4
368	6.2	13.3	7.1	114.5	86.5
371	5.2	11.3	6.1	117.3	42.2
386	5.7	11.5	5.8	101.8	484.0
657	5.1	11.7	6.6	129.4	31.4
673	4.6	11.7	7.1	154.3	930.2
695	4.5	11.1	6.6	146.7	524.4
692	4.5	11.2	6.7	148.9	630.0
Total	40.8	91.3	50.5		3871.1
Average	5.1	11.4	6.3	123.8 Table III	

TABLE III  
AVERAGE PERCENTAGE INCREASES IN FIBRE LENGTH, FIBRE DIAMETER, TENSILE STRENGTH, GREASE, AND AVERAGE FLEECE YIELDS FOR THE DIFFERENT LOTS DURING THE FIVE YEARLY TRIALS

—	No. ewes	Increase in fibre length, %		No. ewes	Increase in fibre diameter, %		No. ewes	Increase in tensile strength, %		No. ewes	Per cent increase in % grease		Raw wool prod., lb.	Clean wool, %	Clean wool prod., lb.
			SE			SE			SE			SE			
Lot I. High Humidity, High Temperature															
1st trial	8	38.4 ± 5.6		8	— 3.7 ± 2.2		7	4.9 ± 4.8		7	76.8 ± 13.7		6.7	58.4	3.9
2nd trial	8	32.4 ± 4.4		8	6.8 ± 1.9		8	— 1.5 ± 3.3		8	25.3 ± 6.0		8.2	61.7	5.0
3rd trial	6	65.6 ± 4.6		7	— 8.1 ± 3.0		7	— 20.0 ± 4.8		7	33.4 ± 4.9		6.5	64.0	4.2
4th trial	7	76.1 ± 7.6		6	— 10.3 ± 2.6		6	— 12.5 ± 5.0		8	7.2 ± 6.8		8.4	44.8	3.8
5th trial	4	54.8 ± 3.6		5	3.0 ± 4.2		5	— 13.5 ± 9.9		4	8.3 ± 14.1		9.2	54.5	5.0
Average		51.9 ± 2.5			— 2.3 ± 1.2			— 7.9 ± 2.4			31.3 ± 4.1		7.7	56.6	4.3
Lot II. Low Humidity, High Temperature															
1st trial	8	47.7 ± 5.6		8	— 1.4 ± 2.2		8	1.6 ± 4.5		7	61.8 ± 13.7		7.6	60.4	4.6
2nd trial	8	41.8 ± 4.4		8	4.3 ± 1.9		8	— 11.6 ± 3.3		6	39.1 ± 6.9		7.6	64.7	4.9
3rd trial	8	64.6 ± 4.0		8	— 5.7 ± 2.8		8	— 17.0 ± 4.5		8	30.7 ± 4.6		6.7	64.3	4.3
4th trial	8	77.8 ± 7.1		8	— 7.3 ± 2.2		8	— 14.5 ± 4.3		8	25.0 ± 6.8		8.8	47.9	4.2
5th trial	4	59.1 ± 3.6		4	3.6 ± 4.8		4	8.8 ± 11.1		4	21.6 ± 14.1		9.7	58.4	5.7
Average		58.1 ± 2.4			— 1.8 ± 1.2			— 8.2 ± 2.2			36.4 ± 4.2		7.9	59.2	4.6
Lot III. Low Temperature, Early Shearing															
1st trial	8	69.1 ± 5.6		8	6.1 ± 2.2		8	4.5 ± 4.5		6	48.8 ± 14.8		6.8	63.8	4.3
2nd trial	8	35.6 ± 4.4		8	1.8 ± 1.9		8	— 10.6 ± 3.3		8	9.2 ± 6.0		7.3	66.1	4.8
3rd trial	8	66.7 ± 4.0		8	— 6.8 ± 2.8		8	— 11.6 ± 4.5		8	43.2 ± 4.6		6.8	63.9	4.3
4th trial	8	91.0 ± 7.1		8	— 7.9 ± 2.2		8	— 11.8 ± 4.3		8	19.6 ± 6.8		8.0	48.8	3.9
5th trial	4	53.7 ± 3.6		4	2.4 ± 4.8		4	11.0 ± 11.1		4	15.9 ± 14.1		8.8	57.6	5.1
5th trial	4	11.0 ± .6*		—	—		—	—		—	30.2 ± 4.2**		—	—	—
Average		65.6 ± 2.7***			— 1.2 ± 1.2			— 5.3 ± 2.2			29.0 ± 4.1***		7.4	60.3	4.4

\* Average fibre length in cm. at end of 5th trial.

\*\* Average percentage grease to clean wool in fleeces at end of 5th trial.

\*\*\* Average of first 4 years.

TABLE III—Continued

AVERAGE PERCENTAGE INCREASES IN FIBRE LENGTH, FIBRE DIAMETER, TENSILE STRENGTH, GREASE, AND AVERAGE FLEECE YIELDS FOR THE DIFFERENT LOTS DURING THE FIVE YEARLY TRIALS—Continued

—	No. ewes	Increase in fibre length, %		No. ewes	Increase in fibre diameter, %		No. ewes	Increase in tensile strength, %		No. ewes	Per cent increase in % grease		Raw wool prod., lbs.	Clean wool, %	Clean wool prod., lbs.
			SE			SE			SE			SE			
Lot IV. Low Temperature, Late Shearing															
1st trial	7	101.4 ± 5.9		7	4.0 ± 2.4		7	-1.9 ± 4.8		6	64.1 ± 14.8		6.9	49.1	3.4
2nd trial	7	62.6 ± 4.7		7	2.2 ± 2.0		7	-30.6 ± 3.5		7	18.0 ± 6.4		7.2	65.4	4.7
3rd trial	7	106.7 ± 4.2		7	-8.5 ± 3.0		7	-18.1 ± 4.8		6	31.2 ± 5.3		7.5	62.8	4.7
4th trial	8	126.1 ± 7.1		8	-13.9 ± 2.2		8	-10.0 ± 4.3		8	33.3 ± 6.8		9.9	45.1	4.5
5th trial	6	10.2 ± .5*		7	-6.7 ± 3.6		7	-4.5 ± 8.4		4	32.5 ± 4.2**		11.0	57.0	6.3
Average		100.1 ± 2.9***			-4.8 ± 1.2			-12.9 ± 2.4			35.7 ± 4.5***		8.3	55.5	4.6
Lot V. Low Plane of Nutrition															
1st trial	7	67.3 ± 5.9		8	-1.4 ± 2.2		8	7.2 ± 4.5		7	49.5 ± 13.7		7.1	57.6	4.1
2nd trial	8	42.0 ± 4.4		7	-1.3 ± 2.0		8	-12.7 ± 3.3		8	14.7 ± 6.0		7.3	64.0	4.6
3rd trial	8	60.6 ± 4.0		8	-6.1 ± 2.8		8	-2.5 ± 4.5		8	39.8 ± 4.6		6.0	69.5	4.2
4th trial	8	97.0 ± 7.1		8	-14.1 ± 2.2		8	-25.2 ± 4.3		8	37.6 ± 6.8		8.3	45.5	3.8
5th trial	4	8.8 ± .6*		4	10.9 ± 4.8		3	-25.4 ± 12.8		4	26.9 ± 4.2**		5.8	56.6	3.3
Average		66.7 ± 2.7***			-4.0 ± 1.2			-9.8 ± 2.2			35.0 ± 4.1***		7.0	58.9	4.1
Lot VI. Medium Plane of Nutrition, Low Protein, Low Mineral															
1st trial	8	73.3 ± 5.6		8	-5.8 ± 2.2		8	-4.3 ± 4.5		7	92.3 ± 13.7		7.6	57.1	4.3
2nd trial	8	47.4 ± 4.4		8	.9 ± 1.9		8	-10.8 ± 3.3		8	11.8 ± 6.0		7.3	63.9	4.7
3rd trial	8	61.9 ± 4.0		8	-2.7 ± 2.8		8	-2.5 ± 4.5		8	33.3 ± 4.6		6.9	70.1	4.8
4th trial	8	123.8 ± 7.1		8	-11.1 ± 2.2		8	-20.3 ± 4.3		8	33.5 ± 6.8		9.9	46.4	4.6
5th trial	4	10.2 ± .6*		4	9.4 ± 4.8		4	37.4 ± 11.1		4	36.9 ± 4.2**		7.8	52.1	4.0
Average		76.6 ± 2.7***			-3.1 ± 1.2			-4.3 ± 2.2			41.1 ± 4.1***		7.9	58.6	4.5

\* Average fibre length in cm. at end of 5th trial.

\*\* Average percentage grease to clean wool in fleeces at end of 5th trial.

\*\*\* Average of first 4 years.

TABLE III.—*Concluded*

AVERAGE PERCENTAGE INCREASES IN FIBRE LENGTH, FIBRE DIAMETER, TENSILE STRENGTH, GREASE, AND AVERAGE FLEECE YIELDS FOR THE DIFFERENT LOTS DURING THE FIVE YEARLY TRIALS—*Concluded*

—	No. ewes	Increase in fibre length, %		No. ewes	Increase in fibre diameter, %		No. ewes	Increase in tensile strength, %		No. ewes	Per cent increase in % grease		No. ewes	Raw wool prod., lb.	Clean wool, %	Clean wool prod., lb.
		SE			SE			SE			SE					
Lot VII. High Plane of Nutrition																
1st trial	8	73.3 ± 5.6		8	.4 ± 2.2		8	— 5.6 ± 4.5		7	66.5 ± 13.7		7	6.9	58.7	4.1
2nd trial	8	44.1 ± 4.4		8	.5 ± 1.9		8	— 9.9 ± 3.3		8	18.5 ± 6.0		7	8.1	63.3	5.2
3rd trial	8	55.1 ± 4.0		8	— 1.4 ± 2.8		8	— 4.5 ± 4.5		8	39.0 ± 4.6		8	7.3	68.4	5.0
4th trial	8	123.9 ± 7.1		8	— 2.1 ± 2.2		8	— 1.3 ± 4.3		7	40.1 ± 7.3		8	11.4	44.5	5.0
5th trial	7	10.8 ± .5*		7	— 7.5 ± 3.6		7	— 9.2 ± 8.4		6	41.3 ± 3.4**		6	8.4	51.3	4.3
Average		74.1 ± 2.7***			— 1.9 ± 1.1			— 6.0 ± 2.3			40.2 ± 4.2***			8.5	57.4	4.8
Lot VIII. Medium Plane of Nutrition, High Protein																
1st trial	8	79.0 ± 5.6		8	— .9 ± 2.2		8	— 7.1 ± 4.5		7	70.9 ± 13.7		8	7.5	59.1	4.4
2nd trial	8	44.0 ± 4.4		8	— 3.3 ± 1.9		8	— 5.4 ± 3.3		8	17.6 ± 6.0		8	8.3	61.1	5.1
3rd trial	8	55.6 ± 4.0		8	— 4.5 ± 2.8		8	— 15.4 ± 4.5		8	27.8 ± 4.6		8	6.5	67.0	4.4
4th trial	8	134.5 ± 7.1		8	— 8.3 ± 2.2		8	— 16.5 ± 4.3		8	33.1 ± 6.8		8	11.2	45.6	5.1
5th trial	6	9.2 ± .5*		6	— 12.4 ± 3.9		4	— 28.3 ± 11.1		4	53.5 ± 4.2**		4	8.0	46.4	3.7
Average		78.3 ± 2.7***			— 5.5 ± 1.1			— 13.0 ± 2.2			36.3 ± 4.1***			8.1	56.9	4.5
Lot IX. Medium Plane of Nutrition, High Minerals																
1st trial	8	78.7 ± 5.6		8	— 3.2 ± 2.2		8	— 1.3 ± 4.5		7	68.3 ± 13.7		8	6.4	60.4	3.8
2nd trial	8	40.9 ± 4.4		8	— 2.1 ± 1.9		8	— 8.4 ± 3.3		8	26.0 ± 6.0		8	7.9	64.2	5.1
3rd trial	8	49.4 ± 4.0		8	— 2.4 ± 2.8		8	— 7.1 ± 4.5		8	35.8 ± 4.6		8	6.6	66.6	4.4
4th trial	8	139.3 ± 7.1		8	— 9.1 ± 2.2		8	— 18.0 ± 4.3		8	39.2 ± 6.8		8	9.7	43.7	4.2
5th trial	6	10.1 ± .5*		7	— 5.4 ± 3.6		5	— 17.9 ± 9.9		5	42.2 ± 3.7**		5	7.8	49.4	3.9
Average		77.1 ± 2.7***			— 4.4 ± 1.1			— 9.9 ± 2.2			41.5 ± 4.1***			7.7	57.4	4.3

\* Average fibre length in cm. at end of 5th trial.

\*\* Average percentage grease to clean wool in fleeces at end of 5th trial.

\*\*\* Average of first 4 years.

TABLE IV  
DIFFERENCES IN AVERAGE PERCENTAGE INCREASES IN FIBRE LENGTH, FIBRE DIAMETER, TENSILE STRENGTH, GREASE AND FLEECE YIELDS BETWEEN TWO GROUPS OF SHEEP MAINTAINED UNDER DIFFERENT EXPERIMENTAL CONDITIONS

—	Diff. in % fibre length increase		Diff. in % increase in fibre diameter		Diff. in % increase in tensile strength		Diff. in % change in % grease		Diff. in raw wool produced, lb.	Diff. in % clean wool	Diff. in clean wool produced, lb.
	SE		SE		SE		SE				
Effect of High Humidity. Low vs. High. Lots II vs. I.											
1st trial	-9.3 ± 7.9	-2.3 ± 3.1	+3.3 ± 6.6	+15.0 ± 20.1	-.9	-2.0	-.7				
2nd trial	-9.4 ± 6.2	+2.5 ± 2.7	+10.1 ± 4.7	-13.8 ± 9.2	+.6	-3.0	+.1				
3rd trial	+1.0 ± 6.1	-2.4 ± 4.1	-3.0 ± 6.6	+2.7 ± 6.7	-.2	-.3	-.1				
4th trial	-1.7 ± 10.4	-3.0 ± 3.4	+2.0 ± 6.6	-17.8 ± 9.6	-.4	-3.1	-.4				
5th trial	-4.3 ± 5.1	-.6 ± 6.4	-22.3 ± 14.9	-13.3 ± 19.9	-.5	-3.9	-.7				
Average	-6.2 ± 3.5	-.5 ± 1.7	+.3 ± 3.3	-5.1 ± 5.9	-.2	-2.6	-.3				
Effect of Temperature. Low vs. High. Lots III vs. I.											
1st trial	-30.7 ± 7.9	-9.8 ± 3.1	+.4 ± 6.6	+28.0 ± 21.0	-.1	-5.4	-.4				
2nd trial	-3.2 ± 6.2	+5.0 ± 2.7	+9.1 ± 4.7	+16.1 ± 8.5	+.9	-4.4	+.2				
3rd trial	-1.1 ± 6.1	-1.3 ± 4.1	-8.4 ± 6.6	-9.8 ± 6.7	-.3	+.1	-.1				
4th trial	-14.9 ± 10.4	-2.4 ± 3.4	-.7 ± 6.6	-12.4 ± 9.6	+.4	-4.0	-.1				
5th trial	+1.1 ± 5.1	+.6 ± 6.4	-24.5 ± 14.9	-7.6 ± 19.9	+.4	-3.1	-.1				
Average	-12.4 ± 3.5	-1.1 ± 1.7	-2.6 ± 3.3	+3.8 ± 5.8	+.3	-3.7	-.1				
Effect of Early Shearing. Late vs. Early. Lots IV vs. III.											
1st trial	-32.3 ± 8.1	+2.1 ± 3.3	+6.4 ± 6.6	-15.3 ± 21.8		+14.7					
2nd trial	-27.0 ± 6.4	-.4 ± 2.8	+20.0 ± 4.8	-8.8 ± 8.8		+.7					
3rd trial	-40.0 ± 5.8	+1.7 ± 4.1	+6.5 ± 6.6	+12.0 ± 7.0		+.1					
4th trial	-35.1 ± 10.0	+6.0 ± 3.1	-1.8 ± 6.0	-13.7 ± 9.6		+3.7					
5th trial	-.8 ± .8*	+9.1 ± 6.0	-15.5 ± 13.9	-2.3 ± 5.9**	-2.2	+.6	-1.2				
Average	-34.5 ± 4.0***	+3.6 ± 1.7	+7.6 ± 3.3	-6.7 ± 6.1***		+4.8					

\* Difference between average fibre length in cm.

\*\* Difference between average percentage grease.

\*\*\* Difference between first 4 years averages.

TABLE IV—Continued

DIFFERENCES IN AVERAGE PERCENTAGE INCREASES IN FIBRE LENGTH, FIBRE DIAMETER, TENSILE STRENGTH, GREASE AND FLEECE YIELDS BETWEEN TWO GROUPS OF SHEEP MAINTAINED UNDER DIFFERENT EXPERIMENTAL CONDITIONS—Continued

—	Diff. in % fibre length increase		Diff. in % increase in fibre diameter		Diff. in % increase in tensile strength		Diff. in % change in % grease		Diff. in raw wool produced, lb.	Diff. in % clean wool	Diff. in clean wool produced, lb.
	SE		SE		SE		SE				
Effect of Plane of Nutrition. Low vs. Medium. Lots V vs. VI.											
1st trial	+ 6.0 ± 8.1	— 4.4 ± 3.1	— 11.5 ± 6.3	+ 42.8 ± 20.1	+ .5	— .5	+ .2				
2nd trial	+ 5.4 ± 6.2	+ 2.2 ± 2.8	+ 1.9 ± 4.7	— 2.9 ± 8.5	0	— .1	+ .1				
3rd trial	+ 1.3 ± 5.7	+ 3.4 ± 4.0	0 ± 6.3	— 6.5 ± 6.5	+ .9	+ .6	+ .6				
4th trial	+ 26.8 ± 10.0	+ 3.0 ± 3.1	+ 4.9 ± 6.0	— 4.1 ± 9.6	+ 1.6	+ .9	+ .8				
5th trial	+ 1.4 ± .8*	— 1.5 ± 6.8	+ 62.8 ± 16.9	+ 10.0 ± 5.9**	+ 2.0	— 4.5	+ .7				
Average	+ 9.9 ± 3.8***	+ .9 ± 1.7	+ 5.5 ± 3.1	+ 6.1 ± 5.8***	+ .9	— .3	+ .4				
Effect of Plane of Nutrition. Low vs. High. Lots V vs. VII.											
1st trial	+ 6.0 ± 8.1	+ 1.8 ± 3.1	— 12.8 ± 6.3	+ 17.0 ± 20.1	— .2	+ 1.1	0				
2nd trial	+ 2.1 ± 6.2	+ 1.8 ± 2.8	+ 2.8 ± 4.7	+ 3.8 ± 8.5	+ .8	— .7	+ .6				
3rd trial	— 5.5 ± 5.7	+ 4.7 ± 4.0	— 2.0 ± 6.3	— .8 ± 6.5	+ 1.3	— 1.1	+ .8				
4th trial	+ 26.9 ± 10.0	+ 12.0 ± 3.1	+ 23.9 ± 6.0	+ 2.5 ± 9.9	+ 3.1	+ 1.0	+ 1.2				
5th trial	+ 2.0 ± .8*	— 18.4 ± 6.0	+ 16.2 ± 15.3	+ 14.4 ± 5.4**	+ 2.6	— 5.3	+ 1.0				
Average	+ 7.4 ± 3.8***	+ 2.1 ± 1.6	+ 3.8 ± 3.2	+ 5.2 ± 5.9***	+ 1.5	— 1.5	+ .7				
Effect of Plane of Nutrition. Medium vs. High. Lots VI vs. VII.											
1st trial	0 ± 7.9	+ 6.2 ± 3.1	— 1.3 ± 6.3	— 25.8 ± 20.1	— .7	+ 1.6	— .2				
2nd trial	— 3.3 ± 6.2	— .4 ± 2.7	+ .9 ± 4.7	+ 6.7 ± 8.5	+ .8	— .6	+ .5				
3rd trial	— 6.8 ± 5.7	+ 1.3 ± 4.0	— 2.0 ± 6.3	+ 5.7 ± 6.5	+ .4	— 1.7	+ .2				
4th trial	+ .1 ± 10.0	+ 9.0 ± 3.1	+ 19.0 ± 6.0	+ 6.6 ± 9.9	+ 1.5	— 1.9	+ .4				
5th trial	+ .6 ± .8*	— 16.9 ± 6.0	+ 46.6 ± 13.9	+ 4.4 ± 5.4**	+ .6	— .8	+ .3				
Average	— 2.5 ± 3.8***	+ 1.2 ± 1.6	— 1.7 ± 3.2	— .9 ± 5.9***	+ .6	— 1.2	+ .3				

\* Difference between average fibre length in cm.

\*\* Difference between average percentage grease.

\*\*\* Difference between first 4 years averages.

TABLE IV—*Concluded*

DIFFERENCES IN AVERAGE PERCENTAGE INCREASES IN FIBRE LENGTH, FIBRE DIAMETER, TENSILE STRENGTH, GREASE AND FLEECE YIELDS BETWEEN TWO GROUPS OF SHEEP MAINTAINED UNDER DIFFERENT EXPERIMENTAL CONDITIONS—*Concluded*

—	Diff. in % fibre length increase		Diff. in % increase in fibre diameter		Diff. in % increase in tensile strength		Diff. in % change in % grease		Diff. in raw wool produced, lb.	Diff. in % clean wool	Diff. in clean wool produced, lb.
		SE		SE		SE		SE			
Effect of Protein. Low vs. High. Lots VI vs. VIII.											
1st trial	+ 5.7 ± 7.9		+ 4.9 ± 3.1		- 2.8 ± 6.3		-21.4 ± 20.1		-1.2	+ 3.3	- .5
2nd trial	- 3.4 ± 6.2		- 4.2 ± 2.7		+ 5.4 ± 4.7		+ 5.8 ± 8.5		+1.0	- 2.8	+ .4
3rd trial	- 6.3 ± 5.7		- 1.8 ± 4.0		-12.9 ± 6.3		- 5.5 ± 6.5		- .4	- 3.1	- .4
4th trial	+10.7 ± 10.0		+ 2.8 ± 3.1		+ 3.8 ± 6.0		- .4 ± 9.6		+1.3	- .8	+ .5
5th trial	- 1.0 ± .8*		-21.8 ± 6.2		-65.7 ± 15.7		+16.6 ± 5.9**		+ .2	- 5.7	- .3
Average	+ 1.7 ± 3.8***		- 2.4 ± 1.6		- 8.7 ± 3.1		- 4.8 ± 5.8***		+ .2	- 1.7	0
Effect of Minerals. Low vs. High. Lots VI vs. IX.											
1st trial	+ 5.4 ± 7.9		+ 2.6 ± 3.1		+ 3.0 ± 6.3		-24.0 ± 20.1		-1.2	+ 3.3	- .5
2nd trial	- 6.5 ± 6.2		- 3.0 ± 2.7		+ 2.4 ± 4.7		+14.2 ± 8.5		+ .6	- .3	+ .4
3rd trial	-12.5 ± 5.7		+ .3 ± 4.0		- 4.6 ± 6.3		+ 2.5 ± 6.5		- .3	- 3.5	- .4
4th trial	+15.5 ± 10.0		+ 2.0 ± 3.1		+ 2.3 ± 6.0		+ 5.7 ± 9.6		- .2	- 2.7	- .4
5th trial	- .1 ± .8*		-14.8 ± 6.0		-55.3 ± 14.9		+ 5.3 ± 5.6**		0	- 2.7	- .1
Average	+ .5 ± 3.8***		- 1.3 ± 1.6		- 5.6 ± 3.1		+ .4 ± 5.8***		- .2	- 1.2	- .2

\* Difference between average fibre length in cm.

\*\* Difference between average percentage grease.

\*\*\* Difference between first 4 years averages.

The standard error of the mean ( $SE_M$ ) for the 97% and 123.8% for the above lots was obtained by dividing the standard deviation for the entire 9 lots by the square root of the number of individuals in each lot or  $\frac{\sigma}{\sqrt{n}}$ .

The standard deviation for the 9 lots was secured by totalling (as shown above) the squares of the deviations from the mean,  $d^2$ , of the 9 lots, dividing such total by the total number of sheep in the 9 lots less one for each lot, and taking the square root of the quotient, *i.e.*,

$$\sigma = \sqrt{\frac{\sum d^2}{n - 9}}.$$

The  $SE_M$  of 97% and 123.9% was  $\pm 7.1$  for each number, as  $\sigma$  and  $\sqrt{n}$  were the same for both lots.

In Table III the percentage increases in fibre length for each year are shown with their respective  $SE_M$ . Below there is shown a weighted average for the five years; its SE was calculated according to the following equation:

$$SE = \sqrt{\frac{\sum (n \times SE_M^2)}{Nn}}, \text{ where } N \text{ is the number of trials.}$$

Thus, for Lots V and VI for the four trials the percentage increase in fibre length was  $66.7 \pm 2.7\%$  and  $76.6 \pm 2.7\%$ .

In Table IV differences between comparable lots are shown for each year and for the five trials combined.

Comparing Lots V and VI to note the "Effect of Plane of Nutrition" in the 4th trial, the figures are  $26.9 \pm 10$ . This is merely the difference between 97% and 123.9% (Table III). Its standard error is  $\sqrt{7.1^2 + 7.1^2}$ .

The same method was used to calculate the average difference in percentage increase in fibre length between Lots V and VI for the five trials. Table IV shows  $9.9 \pm 3.8\%$ , which is the difference between 66.7 and 76.6 in Table III, with the SE equal to  $\sqrt{2.7^2 + 2.7^2}$ .

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Section D

INDEX TO VOLUME XVI

Authors

- Beall, G.**—Analysis of fluctuations in the activity of insects. A study on the European corn borer, *Pyrausta nubilalis* Hubn., 39.
- Bowstead, J. E.**—See Sackville, J. P.
- Bowstead, J. E. and Larose, P.**—Wool growth and quality as affected by certain nutritional and climatic factors. III. Results and conclusions, 361.
- Boyd, Eldon M.**—The interrelation of lipids in the blood of normal rabbits, 31.
- Cameron, D.**—The northern fowl mite (*Liponyssus sylviarum* C. and F., 1877). Investigations at Macdonald College, Que., with a summary of previous work, 230.
- Cameron, J. W. M.**—The reactions of the housefly, *Musca domestica* Linn., to light of different wave-lengths, 307.
- Cameron, T. W. M.**—Investigations on trichinosis in Canada. I. A preliminary survey of the incidence of *Trichinella spiralis* in hogs in eastern Canada, 89.
- Cannon, D. G.**—Some trematode parasites of ducks and geese in eastern Canada, 268.
- Cook, W. H.**—See Sair, L.
- Fallis, A. M.**—See Gwatkin, R.
- Gwatkin, R.**—Anti-*Brucella* serum: guinea pig protection experiments, 353.
- Gwatkin, R. and Fallis, A. M.**—Bactericidal and antigenic qualities of the washings of blowfly maggots, 343.
- Hastings, E. G.**—See Johns, C. K.
- Johns, C. K. and Hastings, E. G.**—Concerning the use of indirect biochemical tests for the diagnosis of chronic contagious mastitis, 6.  
The relation between bacterial numbers and biochemical values in milk from streptococcus-free quarters, 15.
- Kessler, H.**—See Newcombe, C. L.
- Larose, P.**—See Bowstead, J. E.
- Larose, P. and Tweedie, A. S.**—The influence of nutritional and climatic factors on wool growth and quality. II. Laboratory methods used in the measurement of wool characters, 166.
- Mozley, Alan**—The fresh-water mollusca of sub-arctic Canada, 93.
- Munro, S. S.**—The effect of dilution and density on the fertilizing capacity of fowl sperm suspensions, 281.
- Newcombe, C. L., Thompson, S. J., and Kessler, H.**—Variations in growth indices of *Venus Mercenaria* L. from widely separated environments of the Atlantic coast, 1.
- Parnell, I. W.**—Studies on the bionomics and control of the bursate nematodes of horses and sheep. V. Comparisons of the lethal effects of some non-nitrogenous fertilizers on the free-living stages of sclerostomes, 73.
- Richardson, L. R.**—An account of a parasite copepod, *Salmincola salvelini* sp. nov., infecting the speckled trout, 225.
- Sackville, J. P. and Bowstead, J. E.**—The influence of nutritional and climatic factors on wool growth and quality. I. Statement of problem and experimental procedure, 153.

## II —

**Sair, L. and Cook, W. H.**—Effect of precooling and rate of freezing on the quality of dressed poultry, 139.

Relation of pH to drip formation in meat, 255.

**Smallman, B. N.**—See Stanley, J.

**Stanley, J.**—The egg-producing capacity of populations of *Tribolium confusum* Duv. as affected by intensive cannibalistic egg-consumption, 300.

**Stanley, J. and Smallman, B. N.**—A method of determining the mean speed of movement of insects in a mass of flour, 221.

**Strickland, E. H.**—An annotated list of the Diptera (flies) of Alberta, 175.

**Thompson, S. J.**—See Newcombe, C. L.

**Tweedie, A. S.**—See Larose, P.

Section D

INDEX TO VOLUME XVI

Subjects

- Actinonhalas**, 125.
- Amblema**, 122.
- Amnicola**, 116.
- Ancylus**, 113.
- Anodonta**, 123.
- Anodontoides**, 124.
- Anti-Brucella serum**, Protection of guinea pigs by, 353.
- Antigenic qualities** of the washings of blowfly maggots, 343.
- Apatemon gracilis**, Description of, 276.
- Aplexa**, 131.
- Bacterial numbers** and biochemical values in milk from streptococcus-free quarters, 15.
- Bactericidal qualities** of the washings of blowfly maggots, 343.
- Blowfly maggots**, Bactericidal and antigenic qualities of the washings of, 343.
- Brucella abortus**, Protection of guinea pigs from infection with, 353.
- Brucella abortus**, Washings of blowfly maggots antigenic to, in guinea pigs, 351.
- Calliphora erythrocephala**, *C. latifrons*, and *C. vomitoria nigribarda*, Bactericidal action of washings of, 343.
- Campeloma**, 114.
- Copepod**, A parasitic, *Salmincola salvelini* sp. n., infecting speckled trout, 225.
- Corn borer**, European, Fluctuations in the activity of, 39.
- Cynomya cadaverina**, Bactericidal action of washings of, 343.
- Diptera** (flies) of Alberta, An annotated list of the, 175.
- "Drip"** and pH relation in frozen meat, 255.
- Ducks** in Eastern Canada, Some trematode parasites of, 268.
- Echinoparyphium elegans**, Description of, 270.
- Echinostoma revolutum**, Description of, 268.
- Ecological areas** in Alberta and distribution of Diptera, 177.
- Ferrissia**, 114.
- Fertilization**, Artificial, with fowl sperm suspensions, 283.
- Fertilizers**, Effect of non-nitrogenous, on the free-living stages of *Sclerostomes*, 73.
- Fleeces**, Method of sampling, in determination of wool growth and quality, 161.
- Flies** (Diptera) of Alberta, An annotated list of the, 175.
- Fowl mite**, Northern. See *Liponyssus sylviarum*.
- Fowl sperm** suspensions, Effect of dilution and density on the fertilizing capacity of, 281.
- Freezing**, Effect of rate of, on the quality of dressed poultry, 139.
- Fusconaia**, 122.
- Gastropoda**, 95.
- Geese** in Eastern Canada, Some trematode parasites of, 268.
- Guinea pigs**  
Protection of, by anti-Brucella serum, 353.  
Washings of blowfly maggots antigenic to *Brucella abortus* in, 351.
- Hogs** in Eastern Canada, *Trichinella spiralis* in, 89.
- Horses**, Bionomics and control of bursate nematodes of, 73.
- Housefly**, *Musca domestica*, Reactions to light of different wave-lengths, 307.
- Hypoderaeum conoideum**,  
Description of, 274.
- Kainit**, Effect on free-living stages of *Sclerostomes*, 77.
- Lampsilis**, 125.
- Lasmigona**, 124.
- Light** of different wave-lengths, Reactions of housefly to, 307.
- Ligumia**, 125.
- Lime**, Effect on free-living stages of *Sclerostomes*, 86.

**Lipids** in the blood of normal rabbits, Interrelation of, 31.

**Liponyssus sylviarum**

Control, 249.  
Economic significance, 246.  
Geographical distribution, 232.  
Habits, 240.  
Hosts of, in systematic order, 232.  
Life history, 238.  
Morphology, 235.  
Synonymy, 230.

**Lymnaea**, 95.

**Mastitis**, Chronic contagious, Biochemical tests for the diagnosis of, 6.

**Meat**, Frozen, Relation of pH to drip formation in, 255.

**Milk**

from mastitis-infected cows, 8.  
from Streptococcus-free quarters, Relation between bacterial numbers and biochemical values in, 15.

**Mollusca**, Fresh-water, of sub-arctic Canada, 93.

**Moths**, Relation between flight of, and meteorological conditions, 49.

**Musca domestica**, Reactions of the housefly, to light of different wave-lengths, 307

**Musculium**, 119.

**Mya arenaria**, Growth indices of, compared with those of *Venus mercenaria* L., 1.

**Nematodes**, Studies on the bionomics and control of the bursate, of horses and sheep. V. Comparisons of the lethal effects of some non-nitrogenous fertilizers on the free-living stages of Sclerostomes, 73.

**Nicotine sulphate** in the control of northern fowl mite, 251.

**Northern fowl mite**, See *Liponyssus sylviarum*.

**Notocotylus attenuatus**, Description of, 278.

**Parasites**, Some Trematode, of ducks and geese in Eastern Canada, 268.

**Parasitic copepod** infecting speckled trout 225.

**Pelecypoda**, 118.

**Physa**, 111.

**Pisidium**, 120.

**Planorbis**, 105.

**Planorbula**, 110.

**Plasma lipids** of normal rabbits, 32.

**Potash**, Carbonate, muriate, and sulphate of, Effect on free-living stages of sclerostomes, 80, 82, 84.

**Potassium chloride**, Effect on free-living stages of sclerostomes, 81.

**Poultry**

Effect of precooling and rate of freezing on the quality of dressed, 139.  
Infestation of by northern fowl mite, 246.

**Proptera**, 124.

**Psilochasmus longicirratu**s, Description of, 277.

**Pyrausta nubilalis** Hubn., Fluctuations in the activity of, 39.

**Quadrula**, 122.

**Rabbits**, Interrelation of lipids in the blood of normal, 31.

**Red blood cells** of normal rabbits, Lipid content of, 33.

**Salmincola gibber**, Comparison with *S. salvelini* sp. n., 226.

**Salmincola salvelini** sp. n., Description of, 227.

**Salvelinus alpinus** (arctic charr) infested by *Salmincola salvelini*, a parasitic copepod, 225.

**Salvelinus fontinalis** Mitchill, (speckled trout) infested by *Salmincola salvelini*, a parasitic copepod, 225.

**Sclerostomes**, Effect of non-nitrogenous fertilizers on the free-living stages of, 73.

**Sheep**

Bionomics and control of bursate nematodes of, 73.  
Influence of nutrition of, on wool growth and quality, 159.

**Sperm suspensions**, Fowl, Effect of dilution and density on the fertilizing capacity of, 281.

**Sphaerium**, 118.

**Staphylococcus aureus** infection in cows, 10.

**Stephanoprora mergi** sp. n., Description of, 272.

**Streptococcus agalactiae** infection in cows, 8.

**Strophitus**, 122.

**Superphosphate**, Effect on free-living stages of Sclerostomes, 84.

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NUMBER 1

## COLOUR OF MEAT: I. APPARATUS FOR ITS MEASUREMENT, AND RELATION BETWEEN pH AND COLOUR<sup>1</sup>

By C. A. WINKLER<sup>2</sup>

### Abstract

A photoelectric colour comparator, similar to that designed by Bolton and Williams (1), has been constructed and used to compare the colours of meat samples at different pH. Light falls at an angle of 45° on the surface of the sample, and the amount scattered at right angles from the surface in the red, green, and blue regions of the spectrum, defined by standard colour filters in the path of the scattered light, is measured photoelectrically as a percentage of the amount similarly scattered in the same spectral regions from a standard white surface under the same light intensity. The precision of the measurements on meats was  $\pm 0.25\%$  scatter with any one of the three filters.

When samples of pork, beef, and mutton were used, after adjustment of the pH by injections of lactic acid or ammonia, the relation between pH and colour was found to be similar for the three meats, with maximum scatter of red, green, and blue at pH about 5.0–5.5. When uninjected samples of pork were used, scatter in the three spectral regions decreased over the pH range 5.4–6.6, paralleling the changes observed with injected samples within the same pH limits. The visual appearance of the meats is greyish at pH levels acid to the region of maximum scatter and pink in the region of maximum scatter, shading to dark red at higher pH levels. Darkening is paralleled by a decrease in the scatter, while changes in the quality of the colour are accompanied by changes in the ratio of red/green and red/blue.

### Introduction

Good colour in meat, while it might not affect its palatability or nutritive value, is generally recognized and demanded by the consumer. The economic significance of colour and colour stability in meat is therefore obvious.

Of the work that has been done on the colour of meat, that of Brooks (2, 3), Mackintosh and his associates (5, 6, 7), and Heiss and Hohler (4) is probably of greatest interest to the meat packing industry. Much remains to be done, however, and studies of the factors that influence the colour and colour stability of meats, particularly pork and bacon, are being made as part of a program of researches on meats in these laboratories.

Sair and Cook (8, 9) have recently published the results of a study of "drip" from various meats. In the course of their work they observed what appeared to be a relation between pH and colour of the meats. The present paper describes a quantitative study of the relation between pH and colour for pork, beef and mutton.

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Contribution from the Division of Biology and Agriculture, National Research Laboratories, Ottawa. Issued as Paper No. 15 of the Canadian Committee on Storage and Transport of Food.

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## Apparatus

The colour comparator used in these investigations was similar in essential features to that described by Bolton and Williams (1) and is shown diagrammatically in Fig. 1.

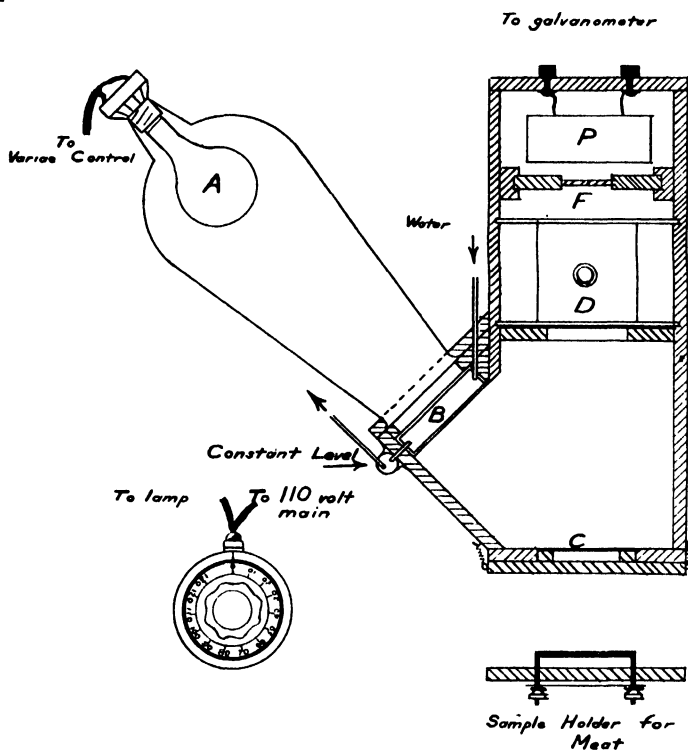


FIG. 1. Diagram of colour comparator for meats.

The light source *A* ("Photoflood" bulb) was mounted in front of a reflector and controlled with a "Variac" transformer. The infra-red radiation reaching the sample was reduced by the passage of the light through water in the glass cell *B*. The light fell at an angle of  $45^\circ$  on the surface of the sample at *C*. The light, scattered approximately at right angles to the surface of the sample, passed through another glass cell *D*, containing a dilute solution of copper sulphate, to remove any infra-red that might be present in the scattered light. Suitable glass colour filters were inserted in the path of the scattered light at *F*, and the filtered radiation was received on a "Photronic" photoelectric cell *P* connected to a sensitive galvanometer.

A magnesium carbonate block was mounted on a holder that could be put in place at *C*. A circular area of two square inches of white surface, covered with a thin glass slide, was exposed. The holder for the test sample consisted of a solid back against which the meat, between thin glass slides, was held in position by a stiff metal plate having a circular hole two square inches in area. The area of meat surface and the area of standard white surface exposed

to the light were therefore equal. Small surface irregularities in the meat sample were eliminated by compressing it slightly between the glass slides.

The method used to measure the colour characteristics of a sample of meat was similar to that described by Bolton and Williams (1). With one of the colour filters and the standard white surface in place, the intensity of the light source was adjusted to give some chosen galvanometer deflection  $d$ . The standard white surface was then replaced by the meat sample, and the galvanometer deflection  $d_1$  obtained. For the colour filter used, the percentage scatter by the sample is then given by  $100 \frac{d_1}{d}$ . The procedure was repeated for other colour filters.

In preliminary tests with the apparatus, several colour filters were used, with a view to obtaining a fairly detailed spectrophotometric analysis of the meat samples. It soon became apparent, however, that sampling errors were too large to justify a detailed colour analysis, and three filters only were finally used. The wave-length ranges over which these transmitted were: blue, 4000 – 4500 Å; green, 4900 – 5800 Å; red, 5750 – 7000 Å. Results are expressed simply as percentage scatter of blue, green and red.

Tests with the apparatus showed that different "photoflood" bulbs and different photoelectric cells gave the same values for the scatter from a given meat sample. It was also found that the time during which the meat was exposed to the intense light, in making measurements with three filters, was not long enough for the colour of the meat to change under the action of the light. With practice, it was possible to make measurements with three filters in about three minutes, and most of this time was taken up in adjusting the light intensity with the standard white surface in position.

The precision of the apparatus has been determined by successive measurements with a given sample of meat, and by measuring the colour of the surfaces along which the knife passes when two samples are prepared from a single piece of meat. By both these methods the value obtained for the percentage scatter could be checked within  $\pm 0.25\%$  with any one of the three filters.

The percentage scatter from two pieces cut from the same muscle of a carcass and brought to the same pH may differ by an amount several times the limit of precision for the apparatus. The magnitude of this sampling error is illustrated in Table I.

TABLE I  
VARIATION IN COLOUR, AT THE SAME pH, FOR DUPLICATE  
SAMPLES FROM THE SAME MUSCLE

Type of meat	pH	Light scattered, %		
		Red	Green	Blue
Pork	5.0	21.1	19.5	16.8
		26.2	23.5	18.2
Beef	5.5	30.0	14.0	14.0
		30.0	14.4	14.8
Pork	5.6	33.0	25.4	19.8
		31.8	24.7	20.0
Pork	5.8	39.5	28.2	24.3
		42.4	26.8	23.5

The values, taken at random from the results obtained with pork and beef, show at once that the precision of the method adopted for measuring the colour was adequate for the investigation.

### Experimental Procedure and Results

Unminced samples (100 to 200 gm. each) from a given muscle of pork and mutton were brought to various pH levels by the method described by Sair

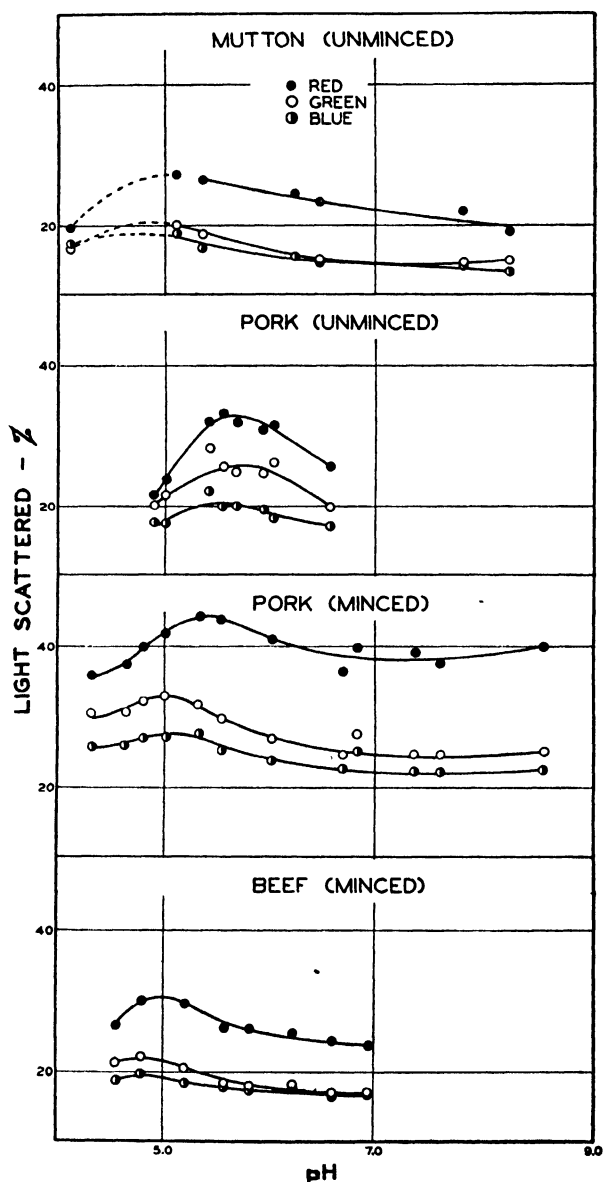


FIG. 2. pH—Colour relation for mutton, pork and beef. Samples injected with lactic acid and ammonia to alter pH.

and Cook (9). It consisted simply of injecting into the meat dilute lactic acid or ammonia solutions of suitable concentrations, followed by 3 to 5 days' storage of the samples at 0° C. A slice was taken from each sample after its removal from storage, and the colour measured. The pH of each slice was determined with a glass electrode. A typical curve for pork is shown in Fig. 2; only one experiment was made with mutton, the results of which are also shown in Fig. 2.

The colour, prior to injection, of the samples used in the experiments described above differed appreciably, and it is probable that the variation in initial colour was reflected, to some extent at least, in the colours developed after injection. It was thought worth while, therefore, to check the results by making experiments in which differences in initial colour were eliminated. This was done by mincing and thoroughly mixing the meat before injections were made. The batch was divided into several portions, and their similarity of colour checked with the colour comparator. They were then injected and stored, with frequent mixing. Typical results for minced pork and beef are represented in Fig. 2.

The range of pH obtainable with the injection method is much greater than that existing normally in carcasses. The pH and colour of cuts from a number of hog carcasses were therefore measured to find out if any relation between colour and pH could be detected for untreated pork. The samples were taken from carcasses in a commercial abattoir, the only factor limiting their choice being that they should be from corresponding muscles of the carcasses. Samples were taken on two different days, at least four cuts at each pH being measured. The average values for the colour at different pH levels are plotted in Fig. 3.

In Fig. 4 are plotted the ratios of the scatters together with the scatter of red from the minced pork. The visual appearance of the samples is also given for the pH regions in which the colour changes were sufficiently marked for recognition; above and below the pH levels at which the meat appeared pink are regions in which it was difficult to describe the colour as it shaded from pink through light brown to grey, or from pink to darker red.

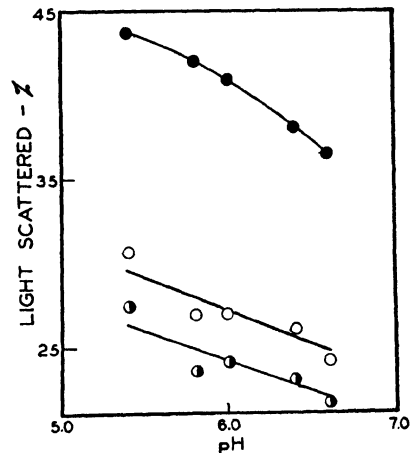


FIG. 3. Average pH—Colour relation for pork from different carcasses. No injections of lactic acid or ammonia. Identity of curves as in Fig. 2.

### Interpretation and Discussion

By examination of the curve for red scatter (Fig. 4) in conjunction with the curves for the ratios, changes in intensity and quality of the colour respectively

can be fairly well described. As a scattering surface becomes lighter in colour, the percentage scatter of all wave bands increases, while if the surface becomes darker the reverse is true. As the trends of the curves for red, green and blue scatters are similar (Fig. 3) the curve for red scatter alone is sufficient to show that from pH about 4.5 to pH about 5.5 the samples become lighter, and beyond pH 5.5, darker in colour. Fig. 4 shows also that there is an increase of approximately 25% in the ratio of red scatter to blue and green scatters

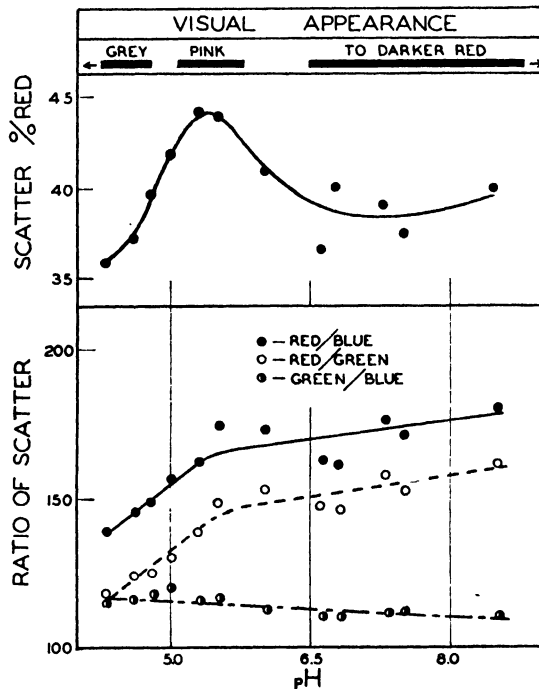


FIG. 4. Relation between pH and ratios of scatters, red scatter and visual appearance (minced pork).

over the pH range about 4.5 to 5.5, paralleling the visually observed change in composition of the colour from grey through light brown to the normal pink colour. From pH 5.5 to 8.5 the ratios of red/green and red/blue increase by only about 8%, and the change over this pH range is mainly in intensity, rather than composition, of the colour. The ratio of green/blue (Fig. 4) is almost constant over the entire range of pH, and is therefore of little or no value in determining the nature of the colour changes.

It is apparent from the curves in Fig. 2 that for meat from a given muscle there is a relation between pH and colour of pork, beef and mutton. The general shape of the pH-colour curves is the same for the three meats, and although it is not evident from Fig. 2, other experiments with pork and beef that gave curves quite similar to those shown, indicate that the pH for maximum scatter from these two meats varies between 5.0 and 5.5. There seems,

then, to be no essential difference in the pH-colour relations for pork and beef, and it is not unlikely that the similarity applies also to mutton, although this should not be definitely concluded from the single experiment.

Four curves for unminced pork, similar to the one shown in Fig. 2, were obtained with samples from the corresponding leg muscles of different animals. The data showed that the amount of scatter at the same pH may differ for different animals by an amount comparable with the change in scatter brought about by alteration of pH in material from a single animal over the entire range investigated. It is therefore evident that factors other than pH are equally or possibly more important in determining the colour of fresh meat from a given animal. Nevertheless, if average values for a sufficient number of samples at each pH are plotted, as in Fig. 3, the relation between pH and colour can be observed with material from different animals.

### Acknowledgment

The author wishes to acknowledge the valuable technical assistance of Mr. E. A. Rooke, Laboratory Assistant, National Research Laboratories.

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# TENDERNESS OF MEAT: I. A RECORDING APPARATUS FOR ITS ESTIMATION, AND RELATION BETWEEN pH AND TENDERNESS<sup>1</sup>

BY C. A. WINKLER<sup>2</sup>

## Abstract

A recording apparatus for estimating tenderness of meat is described. Samples of approximately equal cross section are cut between blunt jaws brought together by a constantly increasing force. The movement of the jaws is recorded on a drum revolving at constant rate, producing a curve on which the co-ordinates of any point can be interpreted in terms of the thickness of sample cut by a given force. By measuring the areas beneath the curves, and applying a correction for variations in initial thickness of the samples, comparative values for the work required to cut the samples can be obtained. The standard deviation of the mean of duplicate determinations on a single sample is  $\pm 3\%$ .

The relation between pH and tenderness was investigated by using samples of pork from three animals and adjusting the pH by injections of lactic acid or ammonia solutions. Toughness was at a maximum at pH about 5.0–6.0; at higher or lower pH levels the meat became progressively more tender. Studies with beef gave similar results, but there was some indication that maximum toughness occurs at a somewhat lower pH. Between different animals the pH at which maximum toughness occurred was more variable in beef than in pork.

## Introduction

As tenderness is one of the most important characteristics of good quality meat, it is being studied in these laboratories as part of the investigations into the storage and transport of meat. Of the factors affecting tenderness, those that have received the most attention from other workers are: position in carcass (2, 4, 5, 8), collagen content (2–5), freezing (2, 8, 9) and storage (1, 2, 6, 9). Both subjective and objective methods have been used for estimating tenderness, and arguments have been advanced in support of both procedures (6, 8). For routine measurements, however, an objective method is almost essential, owing to the difficulty of establishing a panel of suitable judges that would be available as required. An apparatus combining simplicity of design (2) with the advantages of a recording device (10) has therefore been constructed for estimating tenderness.

## Apparatus and Method

A diagram of the recording apparatus is shown in Fig. 1. The sample of meat is placed between the fixed jaw *A* and the movable jaw *B*. The latter is attached to the duralumin lever *C*, which is counterbalanced by the weight *W*. The upper jaw is held by two metal plates between which the lower jaw moves with about 1 mm. clearance on each side. When the slide *F* is pushed to one side in its groove, fine lead shot runs from the hopper *D* into the aluminium pan *E*. A round hole in the slide controls the rate of flow

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of the shot. As the weight of the shot is applied to the lever, the movable jaw approaches the fixed jaw, crushing the meat between them. At the same time, the pen *G* attached to the lever moves downward over a piece of graph paper fastened around the three-inch diameter drum *II*. The drum is geared to a "telechron" motor, which is started simultaneously with the flow of lead shot. This is accomplished by attaching the switch *K* to the slide *F* so that when the slide is opened the switch is closed, and *vice versa*.

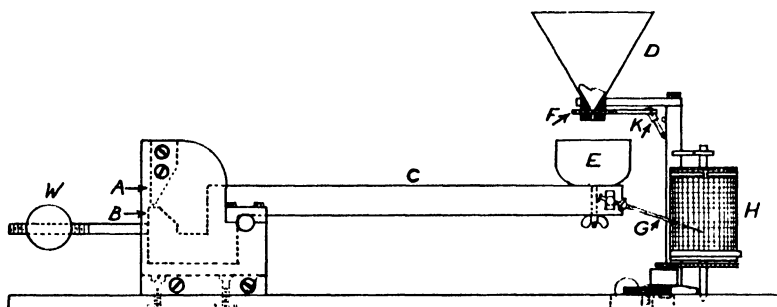


FIG. 1. Diagram of apparatus.

The jaws are similar to those described by Volodkevich (10), and are somewhat blunt, rather than sharp. Their action in severing the sample is presumably similar to that of teeth making a single bite.

To obtain curves convenient for subsequent analysis, the speed of rotation of the recorder drum and the rate of flow of lead shot from the hopper are adjusted to meet the requirements of the material being studied. For beef, pork and bacon it has been found satisfactory to use a rate of flow of shot of about 35 gm. per second, and a drum speed of about one revolution in four minutes. The rate of flow of the shot is constant within  $\pm 0.5$  gm. per second. With lubrication of the bearing by which the lever is mounted, and accurate adjustment of the counterweight, a weight of 3 to 5 gm. is sufficient to move the lever from a resting position. The errors from variation in rate of flow of the shot and from starting friction are negligible in comparison with sampling errors.

The sample of meat to be tested is trimmed to a length of about 4 cm., a thickness of approximately 1 cm. and such a width (about 1.5 cm.) that it fits snugly between the plates on either side of the jaws. Experiments have shown that the width can be 2 to 3 mm. less than the distance between the limiting plates without detectable error, probably because the meat is spread against the plates almost as soon as the cutting action of the jaws begins. The sample is so prepared and inserted between the jaws that it is cut transversely to the direction in which the fibre bundles lie, *i.e.*, across the "grain" of the meat. Care is taken to avoid obvious striations of connective tissue.

As the weight of the lead shot on the lever increases, and the sample is crushed between the jaws, the pen moves downward over the chart on the

revolving drum to trace a curve similar to those shown in Fig. 2. Each pair of curves in the figure represent duplicate determinations on samples of meat from the same muscle of pork or beef. The co-ordinates of a point on such a curve are determined by the amount the pen has moved downward, *i.e.*, by the amount the lower jaw has approached the fixed jaw, and by the length of time the drum has revolved, from which the force acting to cut the sample can be calculated. Duplicate curves for each sample are started at a common point (*a*, Fig. 2), by adjusting the initial position of the pen. The end points (*b*, Fig. 2) of the curves, *i.e.*, when the jaws are together, will therefore differ by the amount the pen was adjusted to have the curves start at the same point,

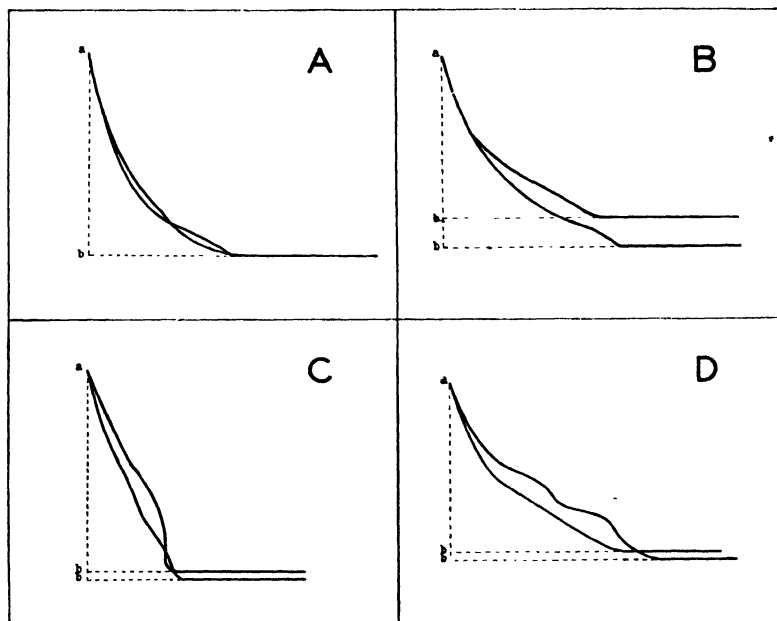


FIG. 2. *Typical curves obtained with the apparatus.*

and the difference between the end points is therefore a measure of the difference in initial thickness of the samples. For example, in *A*, Fig. 2, the duplicate samples were the same thickness, while in *B*, *C*, and *D* they were of different thickness. With the present apparatus there is a slight residual slope at the end point due to flex in the apparatus, but the residual slope is easily identified and seldom interferes with determination of the true end point. If, as with some samples, the curve approaches the end point very gradually, the constant residual slope can be extrapolated back to the point of intersection with the curve in order to obtain the true end point. This is seldom necessary as the error in determining the end point is negligible in comparison with sampling errors.

When the constants of the apparatus are known, the force necessary at the jaws to cut through the sample of meat placed between them can be

calculated. The necessary constants are the rate of flow of lead shot into the aluminium pan on the lever, the rate of revolution of the drum carrying the chart, and the ratio of the lengths of the lever arms. For most investigations, however, comparative values of the cutting force are adequate, and the method adopted for expressing the results has therefore been to estimate, with a planimeter, the area in square inches between the curve and two lines, one drawn along the ordinate axis from the starting point, the other drawn along the abscissa axis at the end point. For a given curve, this area will represent the work done in cutting through a sample, the thickness of which is represented by the ordinate distance from the starting point to the end point. To compare different curves on the basis of work done, all work values must be expressed for unit thickness of sample. Let area  $A$  under the curve for a given sample correspond to an ordinate distance  $d$  (representing the thickness of the sample), and assume that  $d_1$  is the arbitrarily selected ordinate distance representing unit thickness of sample for which the work is to be calculated. Then the work done in cutting unit thickness of the given sample will be  $\frac{d_1}{d} \cdot A$ .

Experiments have demonstrated the validity of this method of correcting all work values to correspond to constant thickness of sample, providing the differences in thickness are not too great. The standard deviation of the mean of duplicate determinations calculated from 20 pairs having mean values varying from 0.8 to 4.6 was  $\pm 0.05$  for samples carefully selected to be free from connective tissue, and varying in thickness by not more than  $\pm 20\%$ . With some practice it is not difficult to cut the samples to a thickness within about half this tolerance. The precision with which tenderness may be estimated by the method is well within the limits of accuracy imposed by sampling errors.

The general shape of a curve obtained with the apparatus is often informative. A rapid change in the slope of a curve (upper curve, Fig. 2C) indicates a "critical" point, at which the force applied suddenly shears resistant fibres. It sometimes happens that more than one rapid change of slope shows on the curve, indicating more than one type of fibre. This is illustrated by the upper curve in Fig. 2D; it is interesting to note that although the samples used to obtain both the upper and lower curve were taken from the same muscle, only the sample corresponding to the upper curve showed heterogeneity of fibre type. The relative positions of the rapid changes in slope indicate the relative toughness of the different types of fibre.

### Experimental Results

Samples of raw leg of pork and loin beef were adjusted to different pH values by injections of lactic acid or ammonia solutions of appropriate concentrations (7). Each sample weighed about 200 gm. and was given 8 to 10 injections of 1 ml., each distributed as evenly as possible throughout its mass. The samples were stored at 0° C. for four days, after which two sub-samples

were cut from each for tenderness measurements. Hydrogen ion concentration was measured on the sub-samples with a glass electrode.

The results of three experiments with pork and beef, each experiment being made with meat from different animals, are shown graphically in Fig. 3. Each point on a curve represents the mean of two determinations, made on different sub-samples from the same sample; the standard deviation of the mean is  $\pm 0.15$  for loin beef and  $\pm 0.23$  for pork, calculated from 21 and 30 pairs of duplicates having mean values varying from 1.0 to 3.4 and 1.3 to 3.6, respectively.

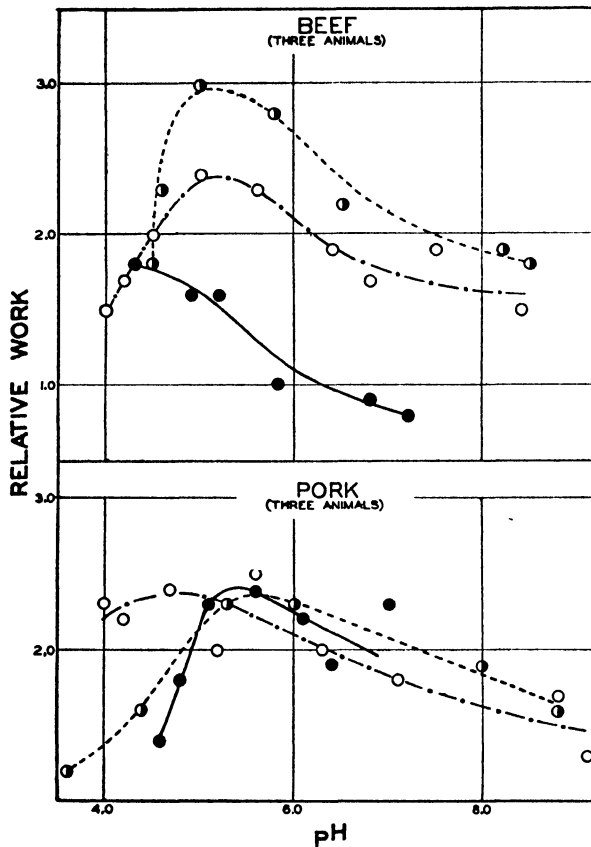


FIG. 3. Relation between pH and tenderness for pork and beef.

### Discussion

The sampling error in these experiments is large in comparison with the precision of the method used for estimating tenderness. With the technique employed, there were three factors that might have contributed significantly to the sampling error, and should, therefore, be considered: (i) variability in initial tenderness of the samples from which the sub-samples for pH and tenderness measurements were taken; (ii) non-uniformity of pH throughout

the sub-sample; (iii) the presence of striations of connective tissue in the sub-samples.

The influence of the initial tenderness on the final tenderness after adjusting the pH was not studied. However, it will be noticed in Fig. 3 that for a single experiment the deviation of any experimental point from the curve is not greater than the standard error for duplicate determinations at a given pH, *i.e.*, for duplicate determinations on a single injected sample of meat. As there is no reason to suppose that the initial tenderness varied more within a sample than between samples, the main error is probably in taking the sub-samples, including the non-uniformity of pH. The variability in initial tenderness would therefore seem to have little or no effect on the results obtained after injection. This is not improbable, because the variations in initial tenderness of samples from a given muscle are not likely to be large.

Non-uniformity of pH is accounted for by the slow rate of diffusion of lactic acid or ammonia through the meat. It would not be reliable to estimate tenderness at the identical position at which pH was measured in the sub-sample, as insertion of the electrodes undoubtedly ruptures some of the tissue. An average value for the pH was therefore obtained from at least two determinations made beside the line along which the sample was cut. It is estimated that this average pH might differ by  $\pm 0.03$  pH from that along the line of cut.

The error that striations of connective tissue would introduce into an estimate of tenderness can usually be avoided with reasonable care in selecting the sub-sample. Occasionally, connective tissue though present is not visible until the sample is cut, and a spurious result is obtained. Two pairs of duplicate determinations were rejected on this account from 55 pairs made during the present investigation.

In general, the curves in Fig. 3 show that the addition of sufficient lactic acid or ammonia to raw pork or beef made the meat more tender. The failure of one curve for beef to show a maximum might be due to experimental error, or possibly the maximum toughness, *i.e.*, minimum tenderness, for the beef used was not attained. The results suggest that for samples at the same pH from different animals, there is a greater variability in tenderness of beef than of pork; the variability observed with beef is, in fact, greater than the change in tenderness brought about by changing the pH within the limits of about 5.5–6.5 generally observed in beef carcasses. There is also some indication that the pH of maximum toughness is lower for beef than pork. More work with both meats would be necessary, however, to determine whether the indicated differences in variability between animals and pH of maximum toughness are significant.

It is not possible to account for the results depicted in Fig. 3 without additional data to supplement the pH—tenderness measurements. It seems unlikely, from the nature of the curves, that hydrolysis of connective tissue around the fibre bundles is responsible, to more than a minor extent, for the observed changes in tenderness. The changes might be associated with

changes in protein-water relations or possibly with increased activity of protein-splitting enzymes.

### Acknowledgment

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# STUDIES WITH A DEFICIENT RATION FOR SHEEP<sup>1</sup>

## I. EFFECT OF VARIOUS SUPPLEMENTS

## II. EFFECT OF A COBALT SUPPLEMENT

By J. E. BOWSTEAD<sup>2</sup> AND J. P. SACKVILLE<sup>3</sup>

### Abstract

Sheep became unthrifty when fed non-leguminous hays and ground oats over a period of seven months. Numerous feed and mineral supplements were fed in an endeavour to prevent the development of the unthrifty symptoms as well as to determine the nutrient or nutrients lacking in the deficient ration. Pasture, calcium and phosphorus, cod liver oil, tankage, linseed meal, bran, and alfalfa meal, when fed as supplements, delayed the appearance of unthriftiness. The feeding of iron and copper proved detrimental. Wheat germ meal did not improve conditions. Alfalfa ash proved to be the best of all the supplements studied in the maintenance of normal thrift. The beneficial effect of alfalfa ash indicated that the deficiency of the non-leguminous ration that caused unthriftiness was one or more of the minerals that were present in the alfalfa hay.

When cobalt was fed as a supplement to a few ewes that had developed the characteristic symptoms of unthriftiness, there followed a rapid increase in weight and improvement in thrift. Chemical analyses of various hays, alfalfa ash, and soil showed that the non-leguminous hays contained only small quantities of cobalt, similar to the amounts contained in New Zealand grass that caused similar symptoms to develop. Alfalfa hay grown on similar soil contained relatively large amounts of cobalt, whereas alfalfa hay grown in the Lethbridge district contained only small amounts of cobalt. The writers suggest that a cobalt problem may exist in Western Canada.

## I. EFFECT OF VARIOUS SUPPLEMENTS

### Introduction

In the last of a series of experiments conducted to determine the extent to which wool growth and quality were affected by certain nutritional and climatic factors (9, 11), there developed among the animals of the nutritional groups symptoms of extreme unthriftiness. The unthrifty condition of the ewes developed among animals fed a liberal ration of oat and prairie hays and ground oats for a period of more than seven months.

The ewes on this ration developed anorexia, lost weight, became anaemic, weak, and 12 out of 16 either died or had to be removed from the ration because of their extreme unthriftiness. In previous years when the same or similar rations had been fed during normal winter periods, no undesirable symptoms developed. Subsequent experience with this and similar rations has proved that the undesirable symptoms develop only after the animals have been fed these rations for long periods.

The recovery of the ewes when fed the usual flock ration containing some leguminous hays and feed supplements suggested that the undesirable symptoms had developed as a result of some nutritional deficiency. The fact that the feeds in the deficient ration were the most common sheep feeds, and the fact that the same symptoms are reported to develop in range sheep following

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prolonged winter periods, made the problem of determining the deficiency of the ration of considerable practical importance.

### Experimental Procedure

Two somewhat similar experiments were conducted, the first from January 1935 to May 1936, and the second from November 1936 to May 1938. During both trials one group of ewes was fed a basal ration similar to the one previously fed and believed to be nutritionally deficient. Other groups were fed the same basal ration plus certain supplements, which there was reason to believe might contain the nutrient or nutrients deficient in the basal ration. In each trial also there was one group of ewes fed a ration of alfalfa hay plus ground oats.

The rations fed were as follows:—

1st trial	2nd trial
Jan. 1935 – May 1936	Nov. 1936 – May 1938
Lot 1. Basal ration (non-leguminous hays and oats).	Lot 1. Basal ration (non-leguminous hays and oats).
2. Basal ration plus pasture.	2. Basal ration plus wheat germ meal.
3. Basal ration plus Ca and P supplements.	3. Basal ration plus linseed meal, wheat bran and tankage.
4. Basal ration plus Ca, P, Fe, and Cu supplements.	4. Basal ration plus alfalfa meal.
5. Basal ration plus Ca, P and cod liver oil supplements.	5. Basal ration plus alfalfa ash.
6. Alfalfa hay and oats.	6. Alfalfa hay and oats.

The hays were fed *ad lib*. The ewes were not forced to consume all of the oat hay allowance, the coarser and more unpalatable portions being considered as unavoidable waste. No hay wastage was allowed for alfalfa, while a very small wastage was allowed for non-leguminous grass hays.

The ground oats, as well as the oat and supplement mixtures, were fed in equal quantities to all lots. The concentrate allowance was increased during the suckling period; it was also increased to those groups losing weight and not consuming normal quantities of roughage.

All ewes were started on test after they had become nutritionally uniform on pasture and then on a common non-leguminous ration for a short period. All ewes remained on their experimental feed till they had been sheared and lambed 18 or 19 months after the trial commenced, or until they were removed from the experiment because of extreme unthriftiness.

Data were obtained on weight, feed consumption, reproduction, wool and any abnormal performance of the ewes.

## Results

Graphs of Figs. 1 and 2 show the weights of the individual ewes during the first and second trials respectively. Average reproduction data are shown in Table I. The results for each group are discussed separately.

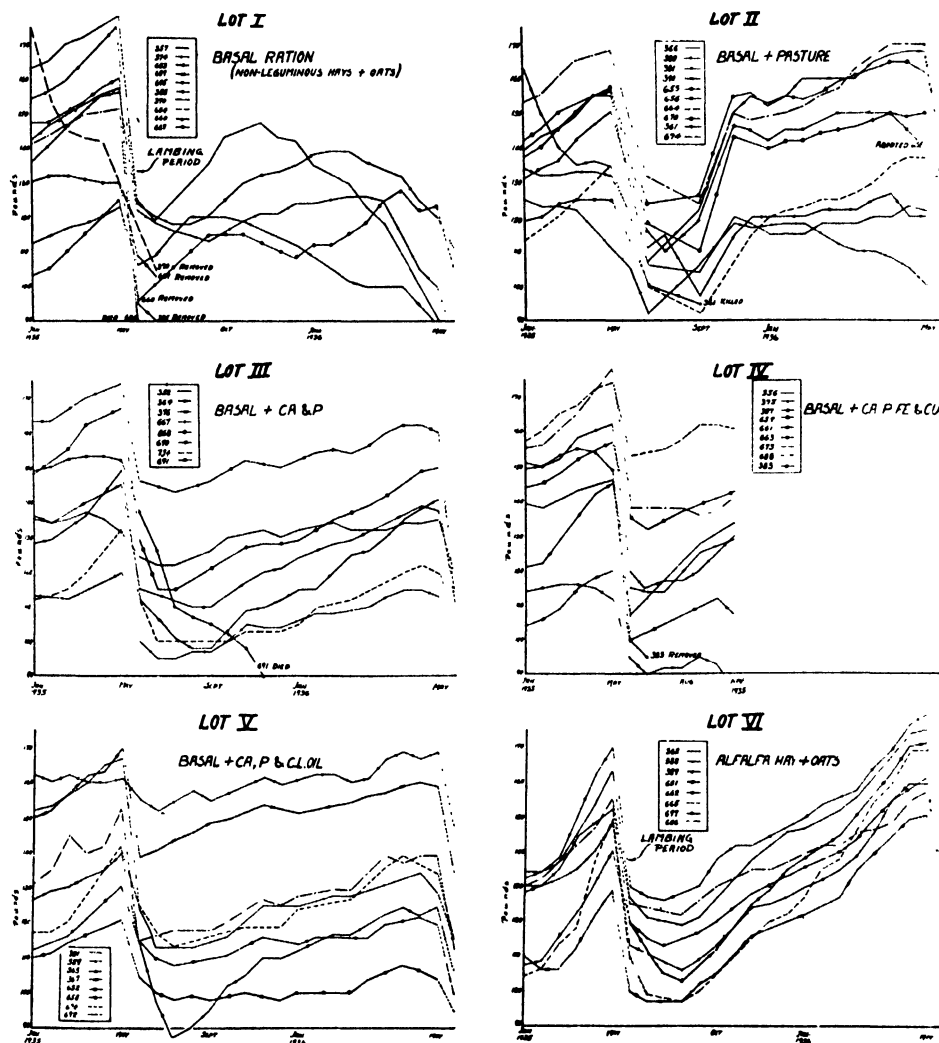


FIG. 1. Graphs showing changes in weight of ewes during 1935-1936.

### Basal Ration

Oat and timothy hays plus  $\frac{1}{2}$  lb. ground oats daily were fed throughout most of the trial. In the first trial five of the ten ewes that began the trial either died or had to be removed from the experiment before seven months had elapsed. These five ewes weighed an average of 47 lb. less at the time of death or removal than they did at the commencement of the trial. The

five remaining ewes continued on the experimental ration till they had lambed after being on test for about 17 months. Four of these ewes had lost an average of 45.1 lb., while one gained 2.3 lb.

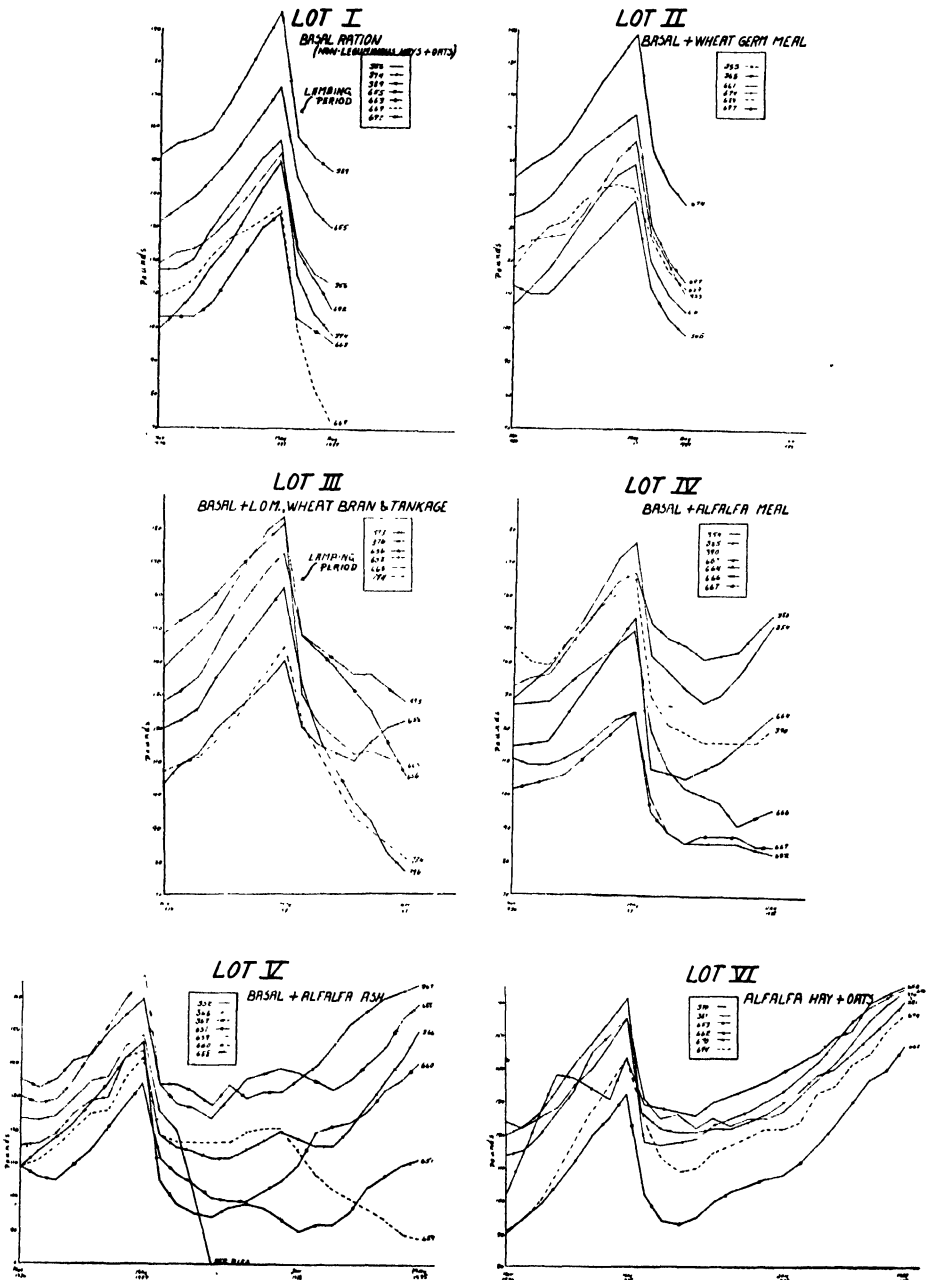


FIG. 2. Graphs showing changes in weight of ewes during 1936-1938.

TABLE I  
REPRODUCTION DATA, 1935-1936 AND 1936-1938 EXPERIMENTS

Lot No.	Ration and supplement	Number of ewes						Vitality at birth			Per cent of living lambs		
		Bred	With- drawn	Died	Aborted	Lambd	No. lambs dropped	Strong, %	Weak, %	Dead, %	Died, %	Weaned, %	
1935 Lambing data													
I	Basal	10	3*	2*		9	13	69	23	8	67	33	
II	Basal + pasture	10				10	16	81	6	13	46	54	
III	Basal + Ca and P	8		1*		8	10	80		20	25	75	
IV	Basal + Ca and P and Fe	9		1*	1	8	13	31	46	23	70	30	
V	Basal + Ca and P and CLO	9				7	10	80	10	10	44	56	
VI	Alfalfa hay	8				8	12	92		8	18	82	
1936 Lambing data													
I	Basal	5	1*			3	4	25	75		75	25	
II	Basal + pasture	10			1	9	12	67	25	8	27	73	
III	Basal + Ca and P	7				7	9	100				100	
IV	Basal + Ca and P and Fe	**											
V	Basal + Ca and P and CLO	9		1		8	12	75	17	8	9	91	
VI	Alfalfa hay	8				8	14	100				100	

\*After lambing.

\*\*Discontinued.

(Continued on Page 20)

TABLE I—*Concluded*  
REPRODUCTION DATA, 1935-1936 AND 1936-1938 EXPERIMENTS—*Concluded*

Lot No.	Ration and supplement	Number of ewes					Vitality at birth			Per cent of living lambs	
		Bred	With-drawn	Died	Aborted	Lambd	No. lambs dropped	Strong, %	Weak, %	Dead, %	Weaned, %
1936-38											
1937 Lambing data											
I	Basal	7	7*			7	12	100	11		25
II	Basal + wheat germ meal	7	7*			6	9	89			33
III	Basal + tankage, etc.	7	7*			7	8	100			75
IV	Basal + alfalfa meal	7	7*			7	10	100			70
V	Basal + alfalfa ash	7				7	10	75	25		70
VI	Alfalfa hay	7				7	13	85	15		84
1938 Lambing data											
I	Basal	**									
II	Basal + wheat germ meal	**									
III	Basal + tankage, etc.	**									
IV	Basal + alfalfa meal	**									
V	Basal + alfalfa ash	7		1	1	5	6	100			100
VI	Alfalfa hay					6	13	92		8	83

\* After lambing.

\*\* Discontinued.

In the second trial the ewes developed the characteristic unthrifty symptoms after eight months of experimental feeding, and all were removed from the trial after nine months on test. While these ewes lost an average of only 13.8 lb., 9 of their 12 lambs born alive and strong had died from apparent malnutrition.

The results from these two groups fed the basal ration indicate that some deficiency existed to account for the failure to maintain thrift and normal reproductive ability.

#### *Basal Ration during the Dry Feeding Period and Pasture during the Summer*

Because the ewes became unthrifty only after a prolonged feeding of the basal ration, it was believed that green grass probably contained the nutrients deficient in the basal ration. In the first trial, after the ewes had been fed the basal ration from November 8, 1934 till May 24, 1935, the group was divided into two uniform groups. One group continued on the basal diet, whereas the other group was turned on to a non-leguminous pasture till November 15, 1935, when they were returned to the basal diet. The experiment ended in May 1936 after the ewes had lambed and were sheared.

The pasture proved beneficial. One ewe had to be killed as a result of a leg injury, but nine ewes completed the trial. One of these aborted and the remaining eight lost an average of over 19 lb.

The lambs dropped by the ewes at the end of the trial were much stronger than those dropped by the ewes on the basal diet, but were weaker than the lambs from the ewes receiving the dry supplements or alfalfa.

The 1936 fleece weights were also heavier for the ewes that had pastured in 1935 as compared to those receiving no pasture. The ewes fed alfalfa hay, however, yielded the heavier and stronger fleeces.

The beneficial effect of the non-leguminous pasture indicated that it provided some nutrient or nutrients lacking in the basal ration. The results, however, indicated that this pasture did not provide all the nutrients that were deficient in the basal diet as the ewes on alfalfa hay made better gains, produced stronger lambs, and yielded stronger fleeces.

#### *Calcium and Phosphorus Supplements*

Seven of the eight ewes remained on test during the entire 17-month period. The seven ewes lost an average of 18 lb. during the trial. The ewe that died lost 71.3 lb. in the 10 months on test. The nine lambs dropped by the ewes were strong and all successfully weaned. Calcium and phosphorus apparently caused a beneficial effect in preventing extreme unthriftiness and maintaining reproductive ability, if the results are compared to those secured from the group fed the basal ration. The results indicate that the basal ration must have been deficient in these minerals. Additional deficiencies of the ration are, however, indicated when the performance of the ewes fed the basal plus mineral ration is compared with the performance of the ewes fed alfalfa hay, as significantly better growth, reproduction, and thrift are shown by the latter group.

### *Cod Liver Oil*

As the unthrifty symptoms that developed in the ewes on the basal ration might possibly have been due to a vitamin A or D deficiency, a group of ewes was given 10 cc. of (1% free acid) Poultry Cod Liver Oil twice weekly in addition to the calcium and phosphorus supplements. One ewe died after 13 months on test. The remaining eight ewes in this lot lost an average of 15.6 lb. while on test, compared to 18 lb. for those ewes not fed the cod liver oil. The eight ewes in this lot dropped 12 lambs in 1936, 11 of which were raised to weaning age. The slight beneficial effects of cod liver oil was not significant, indicating that the deficiency of the basal diet was not either of the vitamins contained in the cod liver oil.

### *Iron and Copper Supplements*

Weekly doses of 0.35 gm. of iron and 0.07 gm. of copper in the form of iron chloride and copper sulphate were fed in addition to the basal ration and calcium and phosphorus supplements. Deleterious effects began to be noted after five months on test. One ewe aborted at that time. One ewe died in less than seven months. While the remaining seven ewes lost an average of only 3 lb., their lamb crop was adversely affected. Of the 13 lambs dropped in 1935 by the eight ewes, 3 were born dead, 7 died soon after birth, and only 3 survived to five months of age. Seven of the nine fleeces were graded as tender, while only one fleece was graded tender in the lot not fed the iron and copper supplements. The deleterious effects may have been due to a poisonous effect of an excess of iron or copper as indicated by the reports of Deobald and Elvehjem (6) and Broughton and Hardy (5).

The results obtained by the feeding of calcium, phosphorus, iron and copper, and vitamins A and D in the cod liver oil suggested that while some beneficial effects were obtained with the calcium and phosphorus, a deficiency was still apparent. The 1936-1938 trial was outlined to find if possible a feed supplement that would provide the apparent deficiency of the basal ration, and indirectly lead to the discovery of the specific deficient nutrient or nutrients.

### *Wheat Germ Meal*

Wheat germ meal was used as a supplement to the basal ration because the complex of life-promoting substances contained in the germ of seeds would possibly include the deficient nutrient. A  $\frac{1}{2}$ -lb. portion of a mixture of 60% ground oats and 40% wheat germ meal was fed throughout the trial. No beneficial effect was noted. The ewes fed on wheat germ meal lost an average of 13.7 lb., while those on the basal ration lost 13.8 lb. Six of the nine lambs dropped by the ewes on wheat germ meal died before weaning, while nine of the twelve dropped by the ewes on the basal ration died. Both the group on wheat germ meal and that on the basal ration were discontinued on August 14 because of their apparent unthrifty condition and loss of lambs.

### *Linseed Meal, Tankage, and Wheat Bran*

Linseed meal and tankage were fed as conditioners or as tonics and to provide, if present, the deficient nutrient of the basal ration. The ewes con-

sumed  $\frac{1}{2}$  lb. of a concentrate mixture of 80% oats, 10% linseed meal and 10% tankage with apparent relish for about eight months. After that time, however, the ewes ate their concentrate mixture less rapidly, and after nine months of experimentation it was deemed necessary to replace the tankage with wheat bran to encourage the ewes to eat the  $\frac{1}{2}$  lb. daily concentrate allowance. The appetite was not improved by removing the tankage nor by adding wheat bran to the concentrate mixture, the ewes showing increased symptoms of anorexia. After 13 months of experimentation it was necessary to remove the ewes from the trial because of their continued decline in weight and thrift. At that time one ewe had been removed, five ewes had lost an average of 30 lb., and one ewe made a gain in weight of 23.5 lb. The seven ewes produced eight lambs, six of which were raised to weaning age.

While some benefit was derived from the feeding of linseed meal, tankage, and later wheat bran, as shown by the maintenance of weight and thrift for a longer period of time, the development of anorexia and extreme unthriftiness suggests that some important nutrient or nutrients were still lacking in the ration.

#### *Alfalfa Meal*

The recovery of the afflicted ewes, when placed on a ration containing alfalfa hay and oats, suggested that alfalfa hay contained the nutrient or nutrients deficient in the basal and other non-leguminous rations. A 0.6-lb. portion of a mixture of equal parts alfalfa meal and oats was fed daily to the ewes to determine whether this amount of alfalfa would contain sufficient of the missing nutrient or nutrients to prevent the development of the unthrifty symptoms. The 0.3 lb. of alfalfa meal was equal to  $\frac{1}{10}$  the amount of alfalfa hay fed to the ewes receiving this hay and oats.

The ewes in this group continued to grow and reproduce normally for eight months before four of the seven began to decline slowly in weight. These four ewes lost an average of 36.2 lb. during the 14 months on test, at which time they had to be removed from the trial. At the end of the 14-month period the three remaining ewes had gained an average of 15.5 lb. and appeared normal in flesh and thrift.

The facts that the ewes on the basal ration were removed from the trial at the end of a 9-month feeding period, and that the ewes fed the alfalfa meal supplement remained thrifty and continued on trial for 14 months, were evidence of the beneficial effect of alfalfa meal. However, as the unthrifty symptoms finally developed, alfalfa meal did not completely provide the necessary nutrients, probably as a result of the small amounts of alfalfa meal actually consumed.

#### *Alfalfa Ash*

The question arose as to whether the beneficial effect of alfalfa hay in preventing unthriftiness was due to its mineral or non-mineral constituents. To answer that question a group of seven ewes was fed the basal ration with alfalfa ash added. The concentrate mixture fed to these ewes was 80%

ground oats and 20% alfalfa ash. In the  $\frac{1}{2}$ -lb. portion fed daily, the ewes consumed the equivalent of ash contained in 1 lb. of alfalfa hay, or  $\frac{1}{3}$  the amount consumed by a group of ewes receiving alfalfa hay and oats as their ration. During the latter months the percentage of ash in the concentrate mixture was reduced to 15% to prevent wastage. Increased amounts of this mixture were fed daily.

None of the ewes receiving alfalfa ash developed the characteristic unthrifty symptoms, and all continued on trial till they had lambed, 19 months after the beginning of the trial. While one ewe died following a short illness after a 10-month period, and one aborted after 16 months on test, the remaining five ewes maintained their weight and thrift. No reason for the death or abortion could be determined. The death of ewes in other groups due presumably to a nutritional deficiency occurred only after a long period of decline. As no abortions occurred in the other groups fed less adequate rations, the abortion recorded in this group is regarded as not being due to the ration fed.

The five ewes produced strong lambs at birth and yielded fleeces that were uniformly good, whereas ewes that developed the characteristic symptoms failed to produce thrifty lambs or to yield fleeces that were strong.

The beneficial effect of the alfalfa ash indicated that the nutrient or nutrients lacking in the basal ration and contained in alfalfa hay is or are minerals that remained in the alfalfa ash.

#### *Alfalfa Hay and Oats Ration*

Because the ewes that developed unthrifty symptoms on the basal and other non-leguminous ration recovered when transferred to the alfalfa hay flock ration, it was thought advisable to carry one group of ewes on a ration of alfalfa hay and oats for comparative purposes. While the basal ration was regarded as deficient, the alfalfa-oat ration was considered to be complete. The ewes were forced to eat all the alfalfa fed and received  $\frac{1}{2}$  lb. of ground oats daily except during the period of heaviest suckling when more concentrates were fed.

The ewes actually gained in weight in both the 1934-1936 and the 1936-1938 trials, and produced large and thrifty lamb crops with a lower mortality than any other lot. The eight ewes in the first trial gained an average of 14.2 lb. and the six in the second an average of 17.8 lb.

Alfalfa hay proved to be the most complete of the rations fed. While the ration containing alfalfa ash gave good results, the ewes failed to make the gains of those fed alfalfa hay, no doubt owing to the fact that they received ash derived from only 1 lb. of alfalfa hay.

## II. EFFECT OF A COBALT SUPPLEMENT

### Introduction

The 1936-1938 experiment (reported in Part I) gave evidence that the deficiency of the non-leguminous hay and oat ration that caused the development of the anorexia and unthrifty symptoms was in the ash portion of alfalfa hay.

The mineral or minerals involved were not indicated by the results as no detailed chemical analyses of the feeds or feed supplements were made.

Toward the close of the above mentioned trial several papers were published that suggested that cobalt might be the mineral that was deficient in the basal ration fed in the trials previously reported. Neal and Ahmann (10), in their experiments with calves in Florida, reported that calves developed anorexia and became unthrifty and anaemic on a ration of Natal grass, corn, skim milk powder, cod liver oil and whole milk. The condition was "prevented or cured by cobalt supplementation and was aggravated by the use of iron and copper supplement." Askew and Dixon (1) reported that the results of their experiments with sheep "definitely suggest that bush-sickness is caused by a mineral deficiency and that cobalt, so potent in overcoming the ailment, is probably present in deficient supply."

### Experimental Procedure

At the time it was decided to study the effect of cobalt as a supplement to the basal ration, animals suitable for the study were few.

In the lot receiving the basal ration plus alfalfa meal, four of the seven ewes had developed the characteristic symptoms and had lost an average of 36.2 lb. These four ewes were, therefore, used in the cobalt supplement study.

The group of ewes that was fed the basal ration plus linseed meal, tankage, and wheat bran, had to be removed from their experimental ration 13 months after the trial began, because one ewe had died and five had lost an average of 30 lb. in weight. One ewe had gained in weight. These particular ewes had been fed a mixture of equal parts alfalfa and timothy hays together with oats for one month previous to placing three of them on the basal ration as controls and the remaining three on the same ration plus cobalt.

Five mgm. of cobalt in the form of the chloride in solution was given to each of the ewes daily, as a drench.

Cobalt determinations were made on the hays that had been fed, and upon the alfalfa ash. In order to compare the cobalt content of similar feeds grown in other parts of the province, two samples of other hays were secured from Lethbridge for analysis.

### Results

The accompanying charts (Fig. 3) show the increase in weight that followed the administration of cobalt to the ewes.

The four ewes that had been fed the basal ration plus alfalfa meal, and that had developed anorexia and were unthrifty, gave an almost immediate response when cobalt was fed. Between December 31 and prior to lambing (approximately May 20), these ewes made an average gain of over 43 lb. The ewes exhibited increased appetites, appeared thriftier, and dropped strong and well-developed lambs, but their fleeces showed a weakness in the centre of the fibres.

It will be noted by comparing the growth charts in Part I that no group of ewes showed as rapid gains in weight as did these four ewes fed cobalt. The

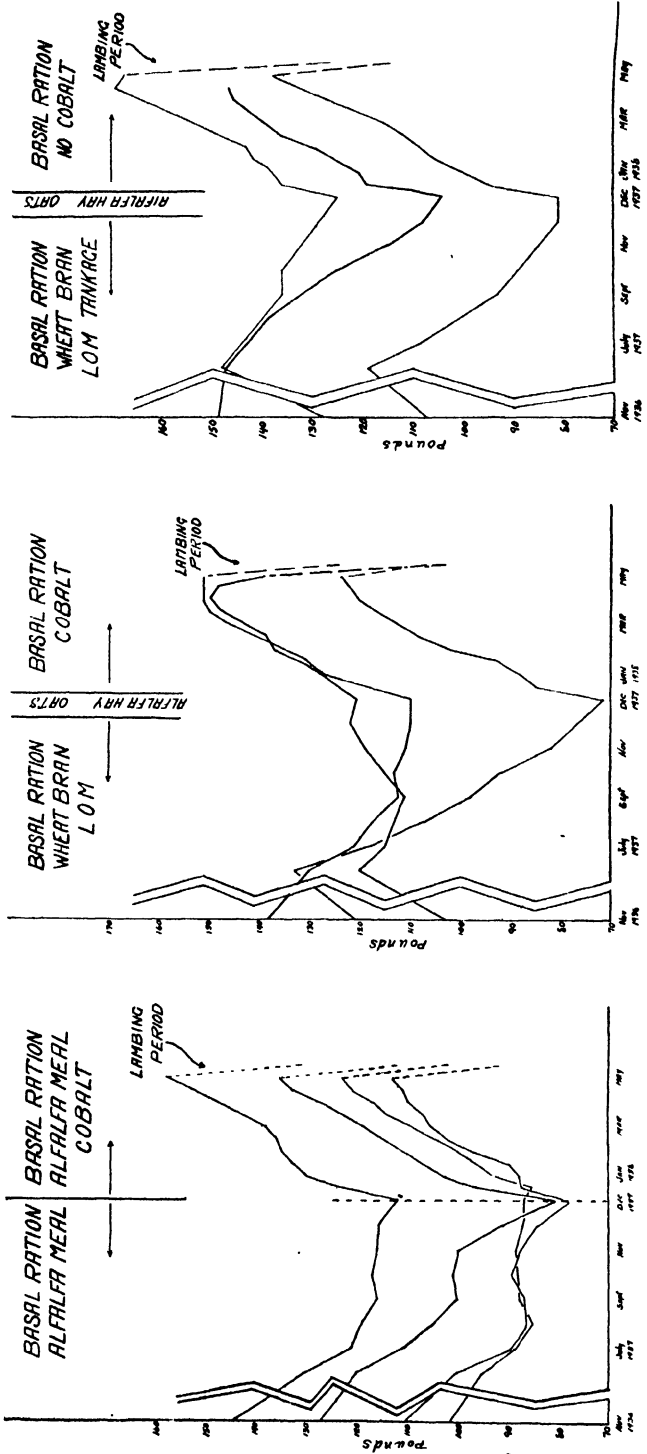


FIG. 3. Graphs showing changes in weight of ewes prior to and after cobalt feeding.

groups of ewes fed alfalfa hay or alfalfa ash supplement gained steadily between December and May but not as rapidly as those receiving cobalt.

The fact that there was no change in the ration in kind and amount prior to and after cobalt was fed, strongly suggests that the recovery of the ewes was due to the cobalt.

The three ewes that had been fed the basal ration plus linseed meal, tankage and wheat bran during 13 months of the trial, followed by one month of alfalfa feeding, also made rapid gains in weight when placed on the basal ration plus cobalt. The fact that the other three ewes in the same lot not receiving cobalt likewise made rapid gains suggests that the gains made by both groups of three ewes may have been caused by the alfalfa hay fed during the month previous and not by the cobalt.

The rapid gains of the three ewes not receiving cobalt may also have been due to consumption of the faeces of the ewes receiving cobalt.

Askew and Josland (3) found in their experiments on the rate of excretion of cobalt by sheep after drenching with similar amounts of cobalt that "practically the whole of the ingested cobalt can be recovered," that "only 2% of the cobalt in the drench appeared in the urine, the remainder, beyond an undetermined amount stored in the organs, being excreted in the faeces." Therefore, as the cobalt requirement is extremely small, and the greater quantity of the amounts fed is excreted in the faeces, the three ewes not fed cobalt may have secured sufficient from the faeces of the ewes receiving cobalt.

Chemical analyses of feeds and soil were made to explain, if possible, the results obtained.

The oat, timothy, and alfalfa hays, as well as the ash used in the 1936-1938 trial were analyzed for cobalt by the Provincial Analyst using the method reported by Kidson, Askew and Dixon (8). The cobalt in parts per million was as follows:

Oat hay	Nil
Timothy hay	.02
Alfalfa hay No. 1	.16
Alfalfa hay No. 2	.16
Alfalfa ash (from alfalfa hay No. 2)	2.00
Alfalfa ash (clinkers from alfalfa hay No. 1)	1.00

The above hays were grown on the University Farm or on similar soil in the surrounding district. The soil upon which Alfalfa hay No. 2 was grown contained 3.40 parts per million of cobalt.

Samples of alfalfa and timothy hays grown in the Lethbridge area were analyzed and the results were as follows:

Alfalfa hay	.012 parts per million
Timothy hay	Nil

Kidson (7) reports that in New Zealand soil, "bush sickness" in sheep occurs in districts where there is less than two parts per million of cobalt. Askew and Maunsell (4) report that in these districts the cobalt content of the pasture is much lower than in areas where bush sickness is not prevalent. Askew and Dixon (2) in their experiments found that legumes take up more cobalt than do the grasses.

The result of these trials and the analyses made suggest that the basal diet was deficient in cobalt. This is indicated by the low amounts of cobalt in the non-leguminous hays as well as by the response that the afflicted animals made to the feeding of cobalt. The superior performance of the ewes fed alfalfa hay was probably due to its higher cobalt content, as shown by its chemical analysis.

The low cobalt content of the Lethbridge alfalfa and timothy hays suggests that sheep in that part of Alberta may be receiving insufficient cobalt for the maintenance of normal growth and thrift.

### Acknowledgment

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## COLOUR OF MEAT: II. EFFECT OF DESICCATION ON THE COLOUR OF CURED PORK<sup>1</sup>

By C. A. WINKLER<sup>2</sup>

### Abstract

In the absence of air, a linear relation, independent of temperature, was observed between moisture loss and colour change. The change was mainly one of intensity, and was reversible. In air, irrespective of its humidity, changes in both intensity and quality of colour occurred, but no definite relation was found between changes in humidity and colour quality for different samples. In saturated air, intensity changes became complete in the early part of the storage period, but the enhanced changes in air of lower humidity continued to increase. No influence of temperature on the rate of colour change was observed in dry air or in air of 60% relative humidity.

### Introduction

The two more important factors contributing to the surface discolouration of meat are methaemoglobin formation and desiccation. The formation of methaemoglobin on the surface of bacon has received considerable attention, notably by Brooks (1), but the effect of desiccation on the colour of this meat seems not to have been quantitatively investigated. Some results that were obtained during a study of this problem are given in the present paper.

### Apparatus

Colours were measured with the photoelectric colour comparator previously described (4). With this instrument the amount of light scattered at right angles to the surface of the sample in the blue, green, and red portions of the spectrum is measured and expressed as a percentage of the amount similarly scattered in the same spectral regions from a standard white surface under the same light intensity. The spectral regions are defined by standard colour filters in the path of the scattered light. The filters used in the present work transmitted the following wave lengths: Blue, 4000–4500Å; Green, 4900–5800Å; Red, 5750–7000Å.

### Experimental Methods and Results

#### *Colour Change by Vacuum Drying*

Moisture is lost at a greater rate from the surface than from the interior of a slice of bacon when drying occurs. Therefore, to make a quantitative

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<sup>2</sup> Biophysicist, Food Storage and Transport Investigations.

study of the relation between change in surface colour and amount of desiccation of sliced bacon, it is necessary either to assume that the moisture lost from the surface layer is directly related to the total moisture loss, or to use very thin slices. There is no reason to suppose the first alternative to be a valid assumption, and the second alternative is difficult to adopt experimentally. By the use of minced bacon, however, the average colour change corresponding to a given loss of moisture throughout the sample can be obtained, and minced bacon has therefore been used in several of the experiments to be described.

Weighed samples (about 50 gm. each) taken from the same batch of minced, unsmoked bacon were dried in *vacuo* at 0° C., 10° C., and 25° C. Each

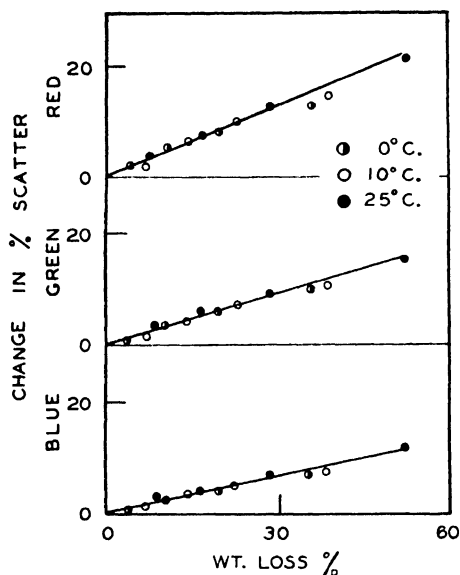


FIG. 1. Relation between colour change and moisture loss in *vacuo*.

sample was periodically weighed, thoroughly mixed and its colour measured. A plot of the results for a typical experiment is shown in Fig. 1, from which it is evident that the relation between colour change and moisture loss is linear and independent of temperature. The change in colour is mainly one of intensity rather than quality; this is inferred from the fact that a decrease of about 43% in scatter of blue, green or red, corresponding to a 50% moisture loss, is accompanied by not more than 6% change in the ratios of scatters, *i.e.*, red/green, red/blue, or green/blue.

The colour change caused by vacuum drying was found to be reversible when partially dried samples of sliced or minced bacon were placed in an atmosphere of water vapour at 10° C. or 25° C. Typical results are shown in Table I.

TABLE I  
REVERSIBILITY OF COLOUR CHANGE AFTER DRYING in *vacuo*

Condition	Time of exposure, hr.	Red scatter, %		Green scatter, %		Blue scatter, %	
		10° C.	25° C.	10° C.	25° C.	10° C.	25° C.
Initial colour	—	34.0	33.4	24.2	24.2	22.0	21.2
Moisture loss	2	—	29.8	—	21.5	—	18.0
Moisture loss	12	26.0	27.8	19.0	18.9	18.6	17.8
Moisture gain	12	28.4	29.1	20.0	20.8	19.4	18.6
Moisture gain	24	31.2	—	21.3	—	20.4	—
Moisture loss	16	27.3	—	18.2	—	17.0	—
Moisture gain	54	—	31.0	—	22.5	—	20.2
Moisture gain	96	34.0	—	24.4	—	22.4	—

At 10° C. the reversibility was complete, providing the samples remained in contact with the water vapour a sufficient length of time. At 25° C., formation of bacterial slime on the surface of the meat always preceded complete recovery of the initial colour, otherwise the behaviour was similar to that observed at the lower temperature.

### Colour Change in Air at Different Relative Humidities

In the vacuum drying experiments, the absence of oxygen precluded colour changes due to methaemoglobin formation. If, however, bacon is stored in air at 100% relative humidity, the formation of methaemoglobin should be the only factor responsible for colour change. On the other hand, the changes observed in air at lower relative humidities should represent the combined effects of drying and methaemoglobin formation.

Several experiments were made to determine the nature and relative amounts of the colour changes resulting from oxidation alone, and from oxidation and drying combined. For each experiment, duplicate samples of about 50 gm. each, taken from the same batch of minced, unsmoked bacon, were stored at room temperature in desiccators through which a slow stream of either saturated or dry air was passed. At intervals, each sample was thoroughly mixed and the amount of colour change determined. The results of a typical experiment are plotted in Fig. 2A.

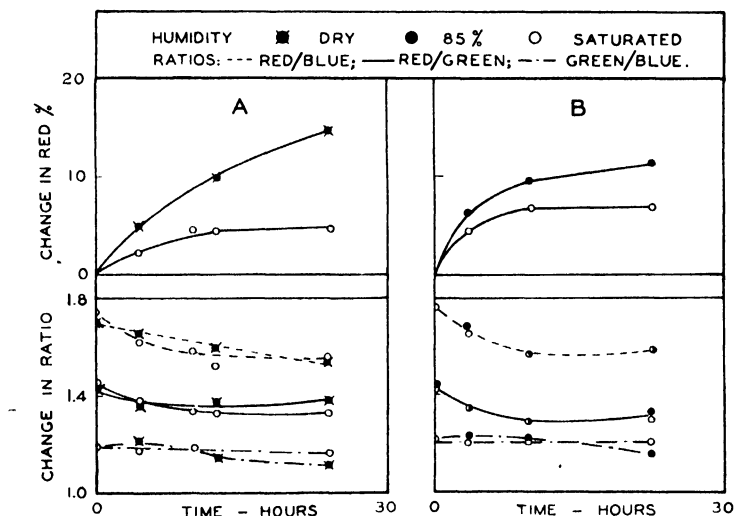


FIG. 2. Effect of storage in dry and moist air on colour intensity and quality.

Unlike storage *in vacuo*, storage in dry or saturated air produced marked changes in quality (upper curves) as well as in intensity of colour (lower curves). In air of 100% relative humidity, change in colour intensity, as inferred from change in red scatter, always attained a maximum value, presumably after methaemoglobin formation had gone to completion. In

dry air, the colour intensity continued to change throughout the storage periods used.

The quality of the colour developed in dry air usually differed somewhat from that developed in saturated air, for samples taken from the same batch of minced bacon. However, the results of a number of experiments with samples from different batches failed to indicate any definite relation between the quality of the colour and relative humidity. This is perhaps to be expected, as the rate of methaemoglobin formation probably depends upon both the rate and extent of drying (2), and these two factors will almost certainly vary from sample to sample.

While the experiments in dry and saturated air were satisfactory for determining the nature and relative magnitudes of the changes in colour of bacon stored under extreme humidity conditions, it was thought desirable to ascertain also the extent to which drying influenced the colour when the humidity approached that used in commercial practice. Experiments were therefore made in which samples of minced, unsmoked bacon were stored at room temperature in slow streams of air conditioned to 85% and 100% relative humidity. The results for a typical experiment are plotted in Fig. 2B. It is evident from the curves for both change in ratio and change in red scatter that during the first few hours' storage at 85% relative humidity, methaemoglobin formation is responsible for most of the observed colour change. Subsequently, however, the effect of drying on colour intensity becomes important, as shown by the increased change in red scatter. With a given sample of bacon, there is little difference in the quality of the colour developed during storage at the two humidity levels.

A few experiments were also made with slices of unsmoked and smoked bacon in air of 60% and 100% relative humidity at 10° C. The results obtained with both these meats in the sliced condition were similar to those shown in Fig. 2B, except that the effect of drying was more marked at the lower humidity and lower temperature. Changes in colour quality were again found to be similar at the two humidity levels.

### *Effect of Temperature*

The effect of temperature on the rate of colour change under drying conditions was studied by storing 50-gm. samples of bacon, both smoked and unsmoked, in a slow stream of dry air at 10° C. and 25° C. Results for typical experiments are shown in Fig. 3. It is apparent that for a given moisture loss from each type of meat, the change in colour intensity is the same at both temperatures. Although slight differences in quality were observed, there was no apparent relation between changes in temperature and colour quality with different samples. Experiments were also made with slices of bacon stored in air of 60% relative humidity at 0° C. and 10° C. Again, no influence of temperature on the rate of colour change could be observed in the temperature interval used.

It is somewhat surprising that the part contributed to the colour change by methaemoglobin formation is not sufficiently different at the different temperatures to be revealed. Presumably the effects due to drying masked the discolouration resulting from methaemoglobin formation, since the changes observed in colour quality indicate that methaemoglobin was formed. It might well be that drying in air of higher humidity would permit a temperature coefficient of colour change to be detected. It might be mentioned,

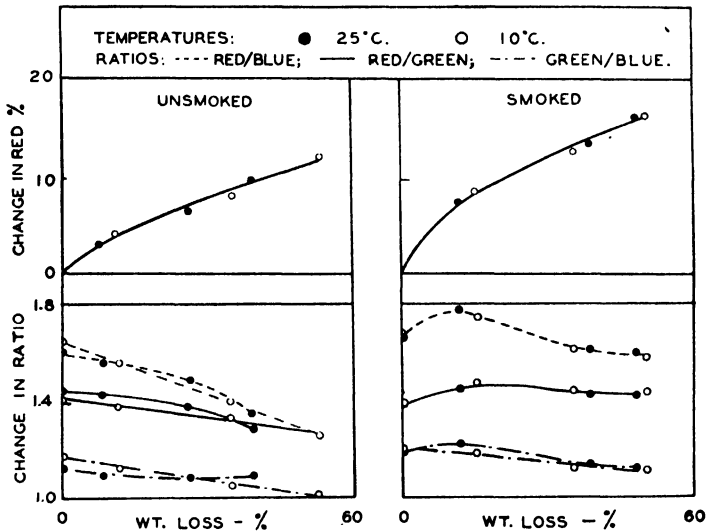


FIG. 3. *Effect of temperature on colour intensity and quality under drying conditions.*

however, that some preliminary studies of the influence of temperature on methaemoglobin formation in minced bacon stored in saturated air gave very variable results. With some samples, temperature appeared to have no influence, while with others the rate of methaemoglobin formation increased two to three times for an increase of 10° C.

### Discussion

Quantitatively the results of this investigation are applicable only to small samples, but certain qualitative generalizations are also possible. When the results are considered as a whole it is evident that in the absence of air and under conditions that prevent desiccation, the colour of bacon should be subject to change only through putrefaction, a conclusion essentially similar to that arrived at by Brooks (1) from other experimental evidence. In the presence of either dry or moist air, however, methaemoglobin formation causes changes in both intensity and quality of the surface colour, the intensity changes but not the quality changes being enhanced by drying.

The effect of desiccation becomes increasingly significant as the storage period is prolonged. Methaemoglobin formation, however, seems to attain to

a maximum value, although a more sensitive instrument than that used might show a slow formation of methaemoglobin following the rapid initial change. According to Brooks (1), methaemoglobin formation is confined to a superficial layer about 5 mm. in thickness, but there is no reason to suppose that the effect of drying is similarly limited, since loss of moisture undoubtedly occurs from the body of the sample. Loss of considerable moisture from the interior of the meat, *i.e.*, in the absence of oxygen, might result simply in changes in the intensity of the internal colour, but there is some evidence (5) that loss of even a relatively small amount of moisture might have profound effects on the colour of freshly cut surfaces.

The reversibility of the changes in colour intensity caused by vacuum drying indicates that the meat pigments are not appreciably denatured by desiccation at ordinary temperatures, and the suggestion of Heiss and Hohler (3) that cathaemoglobin is formed during the drying process seems rather improbable. It seems unlikely also that these results, obtained with minced bacon, can be satisfactorily explained on the assumption that intensity changes caused by drying are due solely to optical effects (2). Concentration of the pigments by removal of water would seem to afford a more satisfactory explanation of both the observed changes and their reversibility.

### Acknowledgment

Grateful acknowledgment is made to Mr. E. A. Rooke for valuable technical assistance throughout the investigation.

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# DEW-POINT HYGROMETER FOR USE AT LOW TEMPERATURES<sup>1</sup>

BY C. A. WINKLER<sup>2</sup>

## Abstract

An apparatus is described in which provision for slow cooling of a metal mirror by circulating over it liquid from a vessel in a thermoregulated bath, and the use of multiple thermocouple elements contained in the mirror, enable the dew-point temperature to be gradually approached and accurately determined. Precise measurements of relative humidity at low temperatures, where the moisture content of the air is small, are therefore possible. A precision of  $\pm 0.5\%$  relative humidity was readily attained at temperatures down to  $-15^{\circ}\text{C}$ .

## Introduction

Accurate measurement of relative humidity, as an essential aspect of its accurate control, is important in relation to the refrigerated preservation of perishable products. Although many instruments, based on several different principles, have been developed for the measurement of humidity (2), many of these are not sufficiently sensitive to permit precise estimation of the moisture content of air at low temperatures, while others of greater sensitivity are generally empirical and require calibration. There is, therefore, an obvious need for an instrument based on sound theoretical principles and capable of accurate determination of relative humidity at low temperatures. These considerations led to the development of the absorption and dew-point instruments described in the present paper.

## Description of Instruments

### *Absorption Hygrometer*

Attempts were made to develop an absorption hygrometer suitable for determining the humidity of air samples drawn from packaged products stored at low temperatures. The apparatus was designed to prevent both access of the sample of air to the desiccant during sampling, and volume changes in the system when the desiccant was brought into contact with the sample. In its final form, the hygrometer was entirely satisfactory in technical details and for use at ordinary temperatures, but the results obtained with it at temperatures below  $0^{\circ}\text{C}$ . were erratic. After a number of unsuccessful attempts to overcome the eccentricities, it was finally concluded that these were due to variable adsorption of moisture on the walls of the test chamber during sampling. Further development of the instrument must therefore be postponed until this difficulty can be overcome.

### *Dew-point Hygrometer*

The two main difficulties encountered in the application of the dew-point principle to the determination of relative humidity at low temperatures are:

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<sup>2</sup> *Biophysicist, Food Storage and Transport Investigations.*

- (i) The observed temperature must yield an accurate estimate of the true temperature of the surface on which dew is deposited, since the dew-point depression per unit change of humidity decreases with the air temperature.
- (ii) The small amount of moisture present in the air at low temperatures increases the time required for a visible deposit of dew to form, and unless the dew-point temperature is approached very gradually its observed value may be considerably below the true value.

Awbery and Griffiths (1) have described a dew-point instrument in which the first of these difficulties is largely overcome by pressing a thermocouple junction against the mirror on the reverse side to that on which dew is deposited. The rate of cooling of the mirror was only partially controlled, however, by regulating the rate of flow of paraffin oil, cooled with solid carbon dioxide, across the reverse side of the mirror. These authors observed a mean difference of 3% in relative humidity between the results obtained with the dew-point instrument and those obtained by the standard gravimetric absorption method.

In the instrument described in the present paper the difficulties mentioned above are both overcome, the first by having multiple thermocouple junctions contained in the mirror and connected in series to increase the thermoelectric potential developed, and the second by providing accurate temperature regulation of the cooling fluid so that the mirror may be cooled as slowly as desired. The accuracy attainable with the apparatus in its present form depends upon the rate at which the dew-point temperature is approached.

The principle of the apparatus can best be understood by reference to Fig. 1. The vessel *C* is filled to the indicated level with a non-freezing solution such as alcohol or ethylene glycol in water. The rest of the assembly, consisting of the liquid-circulating system, dew-point mirror, and thermocouples, is then put into place. In order to circulate the liquid, air is forced through the inlet tube *A* and passes out of the jet *J* below the liquid level. Air bubbles and liquid entrapped between them pass up through the tube *B*, across the under side of the mirror *M* to cool it, and down through the tube *D*, the liquid returning to the vessel *C* while the air escapes at *E*.

The mirror is made of copper, thinly plated with chromium, and has six holes drilled into its edge, each hole to accommodate a thermocouple junction *T* of 40-gauge copper and constantan wires. When in position, the junctions lie about 1 mm. beneath the surface of the mirror, and are held fast and insulated from the apparatus by a thin film of shellac. The mirror can be conveniently mounted by drilling two longitudinal holes in a brass rod, and recessing a portion of this rod as shown in the diagram. It has been found most convenient to immerse the second set of thermocouple junctions in a non-freezing solution in a Dewar vessel, and obtain their temperature with an accurate thermometer. The two sets of junctions are connected in series with a galvanometer calibrated to read directly the temperature difference between them.

The assembly shown in the diagram is immersed slightly beyond the liquid level in *C* in a non-freezing liquid bath provided with a small electric heater, and surrounded by a cooling medium. In practice, an ice formed by freezing a 23% solution of sodium chloride (F.P.  $-21^{\circ}\text{C}.$ ) has been found satisfactory for air temperatures down to  $-15^{\circ}\text{C}.$  Solid carbon dioxide may also be used. With either of these substances a small air space separating the liquid bath from the cooling medium to retard the rate of cooling is an advantage. Cooling by mechanical means has not been attempted but should also be satisfactory. Accurate control of the cooling rate is obtained by manual control of the electric heater in the bath.

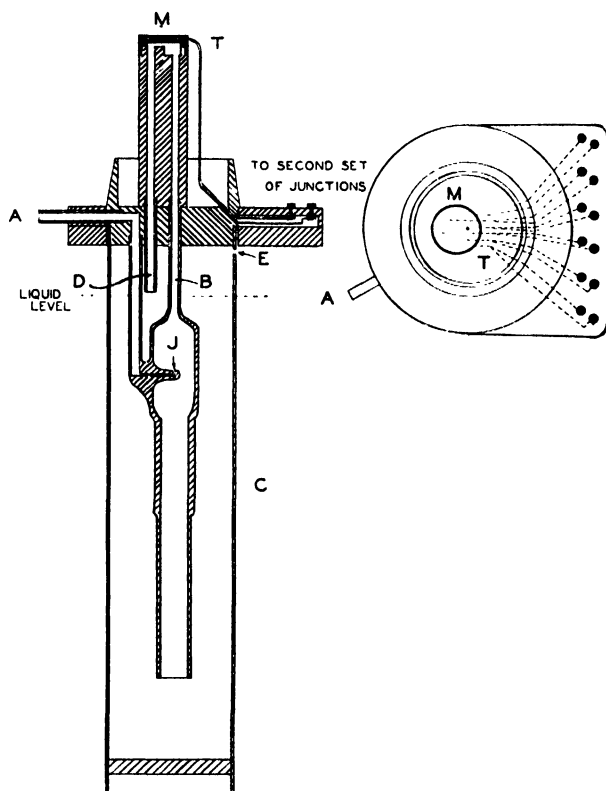


FIG. 1. Diagram of dew-point hygrometer.

An air stream of about two bubbles per second from a jet 0.045 in. in diameter is sufficient for satisfactory circulation of the liquid through the mirror. A small compressor with a safety valve and stopcock in the line to provide control has been found convenient, but a variety of other methods could doubtless be used to obtain the necessary slow stream of air. Displacement of air from a bottle by a liquid under a small head pressure, compressed air, or even a mechanically operated syringe bulb should serve the purpose.

Tests with a manually controlled arrangement of the apparatus at temperatures down to  $-15^{\circ}\text{C.}$ , and over a range of 60 to 95% relative humidity showed that a precision of  $\pm 0.5\%$  relative humidity was readily attained when the dew-point was approached at the rate of about  $2^{\circ}\text{C.}$  per hour. Although precision of this order is quite adequate for most practical purposes, it could doubtless be further increased by approaching the dew-point more slowly. There is no reason to believe that the apparatus would not work equally well at considerably lower temperatures. By providing photo-electric detection of dew formation it should be possible to incorporate the principle into an automatically operating and recording instrument. The development of such an instrument has been undertaken.

### Acknowledgments

Grateful acknowledgment is made to Mr. E. A. Rooke for technical assistance in constructing the apparatus and making tests with it, and to Dr. W. H. Cook for valuable suggestions in its design.

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# THE BIOLOGY OF THE MEADOW NEMATODE *PRATYLENCHUS PRATENSIS* (DE MAN) FILIPJEV 1936<sup>1</sup>

By R. J. HASTINGS<sup>2</sup>

## Abstract

The meadow nematode completes its life cycle in 54 to 65 days—25 to 31 days from the larval stage to the adult, and 29 to 34 days from maturation to the second generation. Eggs are deposited by a single female at the rate of not more than one a day. The largest number of eggs laid by a single female in one place was sixteen, owing apparently to migratory habits. The total number of eggs from a single female could not be determined.

The adult male and female and all larval stages of this species are capable of entering the roots of oats. They are very susceptible to desiccation. No living nematodes were recovered from invaded root tissue that was allowed to dry. In moist excised oat roots, the nematodes remained viable for more than 30 days, but in water the majority died within the same period. A ten-minute immersion of infested oat roots in hot water will destroy the meadow nematode only when the temperature is 120° F., or higher.

## Introduction

Recent investigations by Hastings and Bosher (1) have shown that the meadow nematode, *Pratylenchus pratensis* (de Man) Filipjev 1936, is capable of causing injury to many economically important plants, sometimes reducing growth rates by 50 to 75%. Hence the meadow nematode has to be accepted as an important plant parasite.

Goodey (2) has referred to the life history of the meadow nematode as similar to that of the burrowing nematode, *Rotylenchus similis* (Cobb) Filipjev 1936. He states that the infective stage is probably the first stage larva and that a period of four to five weeks elapses between maturation and the appearance of the second generation.

Experimental evidence was obtained by Hastings and Bosher that the first larval stage is an infective stage, but in addition the evidence proved that the other stages, including adult males and females, can enter oat roots. Both adults and larvae were found in one-day-old oat roots.

## Experimental

Before a study of the life cycle of the meadow nematode was begun it seemed necessary to determine the range in size of each of the several larval stages and adults, and also to determine the ability of each of the stages in the life cycle to enter roots, in order to follow their development within root tissue.

### *Sizes of Adults and Larvae and Their Distribution in a Population from Oat Roots*

Measurements of the length of 223 nematodes were made from a population in water that was obtained by suspending in water a quantity of infested oat roots. The data are presented in Table I.

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TABLE I

THE SIZES OF ADULTS AND LARVAE OF THE MEADOW NEMATODE AND THEIR DISTRIBUTION IN A POPULATION FROM OAT ROOTS

Class	Number	Percentage of total	Length in mm.		
			Minimum	Mean	Maximum
1st stage larvae	74	33.2	.156	.187	.215
2nd stage larvae	37	16.6	.224	.258	.272
3rd stage larvae	17	7.6	.288	.326	.345
Pre-adult larvae	16	7.1	.345	.360	.388
Adult, male	34	15.2	.316	.360	.417
Adult, female	45	20.1	.360	.417	.489

### *Entrance of Adults and Larvae into Oat Roots*

The entrance of distinct stages into oat roots was studied as follows: With the aid of a binocular microscope and capillary pipette, 50 to 75 specimens of each class of the meadow nematode were collected into separate Petri dishes. These suspensions were mixed with powdered peat and in each dish six oat seeds were sown. One week after the seeds germinated, the seedlings were removed and prepared for microscopic examination for the presence of nematodes by washing the roots in running water and clearing and staining them with lacto-phenol and acid fuchsin. All stages were found in the roots.

### LIFE CYCLE OF THE MEADOW NEMATODE

The life cycle of the meadow nematode was studied by following the development of the first stage larvae to maturity in oat roots in relation to time, and by determining the time between entrance of adults into oat roots and the earliest appearance of larvae of the second generation.

#### *A. Development of the First Stage Larvae to Adults*

About 100 first stage larvae were collected in each of three Petri dishes and mixed with powdered peat. Oats were sown in these cultures and the nematodes entered the roots soon after they germinated. The seedlings were kept in the medium for five to six days, and were then transferred to autoclaved soil in individual 4-inch pots. One or more plants were examined at intervals by the lacto-phenol technique. The examinations revealed the presence of adults 25 to 31 days after the larvae entered the roots. The difference between maximum and minimum values is the period that the plants were exposed to infection in the Petri dishes. (Table II.)

#### *B. Time Between Entrance of Adults into Oat Roots and Appearance of Second Generation Larvae*

The method in this experiment was similar to the last, except that adult females and a few males were used as inoculum. Three experiments were conducted, and the results are presented in Table III. In the first experiment, examinations were made more frequently in order to obtain if possible

the rate of egg deposition, the egg output and the egg incubation period. The data from the first experiment revealed that a female residing in the roots for 12 to 17 days laid 12 eggs. No eggs had hatched, hence the egg incubation period is more than 12 days. The data in the second experiment disclosed that 28 to 32 days after the adults entered the roots there were 16-egg nests and only slight evidence of hatching, as 37 females, 235 eggs and only 9 larvae were counted. Most of the 16-egg nests contained no

TABLE II

THE DEVELOPMENT OF FIRST STAGE LARVAE TO ADULTS IN RELATION TO TIME

Date	Days nematodes were in roots		Nematodes found in roots					
	Minimum	Maximum	1st stage larvae	2nd stage larvae	3rd stage larvae	Pre-adult larvae	Adults	Eggs
Feb. 14	4	10	2	4	0	0	0	0
Feb. 25	15	21	0	1	1	0	0	0
Feb. 28	18	24	2	5	5	2	0	0
Mar. 7	25	31	0	1	3	4	2	0
Mar. 11	29	35	0	0	2	2	3	0
Mar. 28	46	52	0	0	0	0	5	2
Apr. 4	53	59	0	0	0	0	1	2

larvae, hence the egg incubation period is more than 16 days. The data in the third experiment showed that 26 to 30 days after the adults entered the roots three females had produced 30 eggs, of which one had hatched. After 33 to 37 days, however, 25 females, 254 eggs, and 56 larvae were counted from the roots examined. In several of the nests there were as many as four larvae, probably four days old. If four days are deducted from 33 to 37 days, the minimum time between entrance of adults and the appearance of larvae would be 29 to 34 days. The life cycle of the meadow nematode is thus 54 to 65 days, 25 to 31 days for the first stage larvae to reach adult stage, and 29 to 34 days from maturation to the appearance of a second generation. The life cycle of *P. pratensis* is apparently somewhat similar to *Ditylenchus radiculicola*, the root gall nematode, which was recently studied by Goodey. He reported that the life cycle took 56 to 64 days (3).

The data contained in Table III also showed that the maximum egg output of any single female at any time throughout the duration of the experiments was always less than the age of the plants in days, which suggests that the female nematode does not lay more than one egg a day.

#### OBSERVATIONS CONCERNING SOME OF THE HABITS OF THE MEADOW NEMATODE

The nematodes that entered oat roots from peat cultures were invariably found in the older parts of the tissue, seldom in or near the root tip. The larvae were headed generally in the direction of the root tip. The adults

were headed in all directions. The nematodes are vagrant but do not seem to travel very far in the root tissue. The course travelled by females could be followed by the position of the eggs. The eggs are usually grouped together, but occasionally they may be spread out. In this case the most distant egg was not more than 2 mm. from the parent. After depositing 15 to 16 eggs, the female usually migrates. Migration occurs via the soil. In peat cultures the nematodes have travelled through  $3\frac{1}{2}$  in. of medium in three days. In soil the migration rate may be as rapid. Owing to this habit of migration, the number of eggs that a single female produces could

TABLE III

TIME BETWEEN ENTRANCE OF ADULTS INTO OAT ROOTS AND APPEARANCE OF EGGS AND SECOND GENERATION LARVAE

Date of examination	Days in roots		Nematodes found in the roots			Number of eggs laid by individual females		
	Min.	Max.	Adults	Eggs	1st stage larvae	Min.	Max.	Average
Series 1								
Jan. 14	0	3	3	2	0	0	2	0.5
Jan. 18	2	7	7	7	0	0	4	1.0
Jan. 21	5	10	8	15	0	0	4	1.8
Jan. 24	8	13	16	81	0	2	9	5.0
Jan. 26	10	15	4	27	0	5	9	6.7
Jan. 28	12	17	4	44	0	9	12	11.0
Series 2								
Feb. 7	0	4	3	0	0	0	0	0
Feb. 9	2	6	102	21	0	0	2	0.2
Feb. 14	7	11	3	2	0	0	2	0.6
Feb. 18	11	15	4	12	0	0	6	3.0
Feb. 21	14	18	3	10	0	0	6	3.3
Feb. 28	21	25	18	117	0	0	12	6.5
Mar. 7	28	32	37	235	9	0	16	7.0
Mar. 11	32	36	7	56	7	0	14	9.0
Series 3								
Feb. 18	9	13	4	12	0	2	6	3.0
Feb. 24	15	19	48	341	0	3	9	7.0
Feb. 28	19	23	1	15	0	15	15	15.0
Mar. 3	22	26	12	126	2	5	15	10.5
Mar. 7	26	30	3	29	1	0	16	10.0
Mar. 14	33	37	25	254	56	0	16	12.0

not be determined. The largest number in a nest, traceable to an individual female, was 16, but reference to Table III shows that at the end of each series of tests there were females with no eggs close to them, indicating that they had migrated from some other position. Observations of a 26-day-old plant definitely substantiate this. The plant had three roots. Root 1 contained no nematodes, but four nests of 7, 13, 13, and 12 eggs respectively. Root 2 had two females with no eggs and another female with nine eggs. Root 3 contained six females but only five nests of eggs.

The eggs in oat roots are usually found in the cortex, generally in an axial position to the root, but eggs in a transverse position were also found, in one instance 3 in a nest of 14. This observation differs from that of Steiner with respect to the meadow nematode in rice roots (4). Steiner reported finding no eggs in a transverse position and suggested that there may be a possible connection between the regular axial position of the eggs and either (a) the mode of deposition or (b) the character of root cells and type of tissue.

#### EFFECT OF DESICCATION ON THE MEADOW NEMATODE

Desiccation proved fatal to the meadow nematode. In one experiment, infested oat roots were air-dried for three days and were then suspended in water; but although there were numerous nematodes in the tissue, none emerged, as all had died. In another experiment, freshly excised infested oat roots were placed in water. In 48 hr. there was an abundance of nematodes in the suspension. The water was allowed to evaporate, and the dish was kept dry for 24 hr., then water was added. None of the nematodes regained their motility; they had died from the effects of desiccation.

#### SURVIVAL IN EXCISED OAT ROOTS AND IN WATER

In excised oat roots that were maintained in water, the meadow nematode survived for a period of four weeks. Infested oat roots were transferred every few days to a different dish containing water, and in each of seven suspensions there was an emergence of the nematodes into the water. On the thirtieth days of the experiment, an examination was made of the nematodes in each suspension, and the number and percentage of living and dead nematodes was determined.

TABLE IV  
SURVIVAL OF THE MEADOW NEMATODE IN EXCISED OAT ROOTS AND IN WATER

Date of suspension of excised infested oat roots	Period nematodes were free-living in water (days)	Number of nematodes in suspension	Number alive	Percentage alive
1st - 6th	24 - 30	112	19	16
6th - 10th	20 - 24	37	18	48
10th - 14th	16 - 20	87	47	53
14th - 18th	12 - 16	28	18	64
18th - 22nd	8 - 12	10	10	100
22nd - 26th	4 - 8	6	6	100
26th - 30th	1 - 4	3	3	100

In the case of those emerging early from the oat roots, four weeks in water usually exhausted the nematodes and caused the death of over 80%. However, even after six weeks there were still a few survivals. The nematodes that remained quiescent within the root and conserved their energy were able to survive longer than those in the free-living condition. The stage of development was not a factor in these survivals.

### MINIMUM LETHAL TEMPERATURE OF THE MEADOW NEMATODE

A 10-minute immersion of infested oat roots in water at 120° F. destroyed the meadow nematode. Immersions of infested roots at lower temperatures failed to kill the nematodes, but delayed their emergence from the roots.

Oat plants were immersed in hot water for 10 min. and the excised roots were afterwards suspended in water for the emergence of surviving nematodes. Observations made on the seventh day of suspension disclosed emergence of survivors from roots immersed at 100° F. and 105° F. but not from roots immersed at higher temperatures. On the tenth day, there was emergence from roots treated at 110° F., and on the fourteenth day, from roots treated at 115° F., but up to the thirtieth day, no nematodes came out of the roots treated at 120° F. Thompson (5) has reported that lily of the valley crowns have been freed from the meadow nematode by immersing the plants for 30 min. at 113° F. In the present study, infested oat roots were immersed at the same temperature for the same period but nematode survival was found.

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# A STUDY OF BACTERIA CONTAMINATING SIDES FOR WILTSHIRE BACON WITH SPECIAL CONSIDERATION OF THEIR BEHAVIOUR IN CONCENTRATED SALT SOLUTIONS<sup>1</sup>

BY E. H. GARRARD<sup>2</sup> AND A. G. LOCHHEAD<sup>3</sup>

## Abstract

Forty microbial types, in which micrococci predominated, were found as representative of pre-curing contamination, including strains resembling organisms found in bacon slime.

Varying degrees of salt tolerance were noted, micrococci showing the greatest ability to grow at higher concentrations. With 25% sodium chloride only two species showed growth. Tests with species reducing nitrate to nitrite at 5% salt concentration showed that as the salt concentration increases, nitrate reduction occurs with a progressively smaller proportion of those showing growth. This suggests that nitrate reduction in curing pickle is a function of the true halophiles rather than of the pre-curing contaminants.

The organisms could be placed in five groups depending upon their salt resistance (ability to survive) in salt solutions and in curing pickle. Much greater resistance to salt was displayed in curing pickle than in salt broths of similar sodium chloride content. Pickle appeared to possess substances tending to neutralize the toxic effect of salt, the action being "protective" rather than "stimulative".

Many types of bacteria constituting original contamination are considered able to survive the pickling process. Although the findings do not point to any pronounced activity of these in pickle, their ability to survive opens the possibility of their becoming active after pickling and contributing to storage defects. The results justify the adoption of measures for the utmost plant sanitation in Wiltshire processing.

## Introduction

The greater part of the bacon exported from Canada is in the form of Wiltshire sides, which are pickled, drained, and baled in Canada and receive further treatment in England. From time to time, sides when received are found to show off-flavour, discoloured areas, or slime, the latter principally on the membrane of the rib tissue. Bacteriological examination of slime may show considerable numbers of micro-organisms, many of which are non-halophilic in nature, despite the fact that the sides have been cured in strong brine and, after draining, have a salt content of some 4 to 5%. This suggests that undesirable bacteria either may be present in the pickle or may contaminate the sides during draining, wiping, and baling operations. The extent of the defects on the sides will depend naturally on their development as affected by temperature, humidity, and time of storage before arrival of the sides on the market.

It is well known that carcasses may be contaminated during operations on the killing floor of slaughterhouses and packing plants and that this contamination may be augmented by subsequent handling of the meat. It has

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been shown by Haines (2) that the initial bacterial load noticeably affects the storage life of the meat. Obviously the storage life will also be dependent upon the extent to which contaminating bacteria have been allowed to develop. The importance of such factors as temperature, relative humidity, and carbon dioxide concentration during storage has been shown by a number of workers, such as Schwartz and Schmid (5), Haines (1), and Scott (6, 7, 8). For the fresh and chilled meat trade, the prevention and control of microbial contamination are thus matters of the greatest concern, and standards of cleanliness are advised to keep contamination by, and growth of, micro-organisms at a minimum.

In Wiltshire processing, involving the curing of sides in concentrated brine under conditions in which bacteria are considered essential agents, the significance of the initial contamination is by no means clear. In our packing plants, hygienic measures are adopted to minimize contamination prior to curing, on the justifiable assumption that micro-organisms contaminating meat, if allowed time and suitable temperature, may cause various types of spoilage. However, there is little or no exact knowledge of the bactericidal or bacteriostatic effect of the high salt content of the curing pickle on contaminating organisms, nor on the more practical questions as to the possible role of such contaminants in the cure, or the ability of certain types to survive the pickling and act as possible agents of spoilage during the subsequent storage period.

Preliminary studies (4) in the analysis of curing pickle have shown the presence in the brine of appreciable numbers of non-halophilic organisms, able to resist, if not multiply in, high concentrations of salt. Observations (unpublished) on the surface load of Wiltshire sides at different stages of the processing showed that organisms able to develop without salt were an important group of bacteria prior to pickling, and likewise comprised a group that definitely increased during the storage of the pickled sides after baling. Taken together, the above sets of findings suggest that deleterious organisms that may contaminate sides prior to pickling, may carry through and cause defects on cured, stored sides. The aim of the present study was to obtain information of a more direct nature on this point.

### Experimental

The main purpose of the experiments here reported was to study the types of bacteria contaminating sides of Wiltshire bacon prior to curing, and to note particularly their ability to tolerate various concentrations of salt, with distinction between capacity for growth and for survival. Samples were taken from sides just prior to pumping and pickling, as being representative of plant contamination preceding the cure. To afford further information and obtain representative types of organisms from various sources of contamination, samples were also taken from freshly slaughtered hogs, from sides in the chill room just before removal to the cutting room (approx. 40 to 48 hr. after killing), and from sawdust, air, and wall scrapings in the chill room.

Surface contamination of the sides was studied by a filter paper impression method, using Whatman No. 3 filter paper cut into squares of 4 sq. cm. and sterilized in Petri dishes in hot air at 120° C. Samples were taken by pressing a square of paper firmly against the meat tissue for 20 sec. with sterile forceps, and then dropping it into a 750-ml. Erlenmeyer flask containing 500 ml. physiological salt solution and 75 gm. broken glass. Various methods of estimating surface contamination of meat have been used. The impression method was selected in preference to those involving scraping, or cutting out measured areas, as it obviated any mutilation of the tissue. From each side, impressions were taken as follows: two from lean flesh, two from centre rib section and one from lower short ribs. To each flask were added 20 squares representing impressions from four sides. Hogs were grouped into lots of four, picked at random as they came from the killing and dressing floor on their way to the cool room. From each group (eight sides) two flasks were prepared. In all, 32 sides were used for sampling, marked so they could be followed to the cutting room.

At the laboratory, flasks were shaken for 10 min. until the filter paper was disintegrated, and the contents of each pair of flasks were mixed in a previously sterilized 2000-ml. flask. From the combined suspension of 40 squares, further dilutions were prepared with physiological saline blanks, and plates were poured using nutrient agar and agar media containing respectively 5, 10, and 15% sodium chloride\*. All plates were incubated at room temperature (approx. 20° C.). Nutrient agar plates were counted after five days, 5% and 10% salt plates after two weeks, and 15% salt plates after three weeks. Similar media were used for the quantitative determination of organisms in sawdust, air, and wall scrapings of definite areas.

### Pre-curing Contamination

Though the main object of the work was to obtain representative types of bacteria for detailed study rather than a systematic quantitative examination of plant contamination, quantitative data illustrating the trend of surface contamination at different times are shown in Table I. In Table II is found a summary of results from the examination of sawdust, wall scrapings, and air.

As expected from an examination of this kind, in which the extent of contamination is varied and accidental direct contact with walls, clothing, etc., may make for great localized differences, variations are noted. The method of sampling involves additional errors due to the possibility of touching the same area twice and of covering more grossly contaminated spots from which contamination may be later reduced in various trimming, cutting, and wiping processes. The results show, however, that sides for Wiltshire bacon may become definitely contaminated with bacteria prior to pickling, within the space of some 40 to 48 hr.

\* In this paper, % sodium chloride = grams per 100 ml. of solution.

The findings suggest that under conditions in which sides are removed, promptly after killing, to a chill room of approximately 32° F., the microbial load at the end of cutting is the result of direct contamination rather than growth. It is possible for sides to leave the chill room with little or no increased microbial load; when increased counts are noted, they are regarded as due largely

TABLE I  
SURFACE CONTAMINATION OF WILTSHIRE SIDES PRIOR TO CURING

Group No.	—	Count per sq. cm. on agar			
		0% NaCl	5% NaCl	10% NaCl	15% NaCl
1	Freshly killed	87	87	19	6
	After cooling	1,110	1,090	450	220
	After cutting	3,890	4,860	2,260	790
2	Freshly killed	3,550	5,350	3,031	750
	After cooling	31,500	40,560	5,150	100
	After cutting	3,750	2,030	1,820	340
3	Freshly killed	630	220	100	50
	After cooling	660	150	50	20
	After cutting	12,130	7,440	1,220	490
4	Freshly killed	150	190	170	40
	After cooling	250	130	70	6
	After cutting	10,810	7,810	2,140	840

to direct contact infection. Table II suggests that air is normally a relatively insignificant source of contamination, whereas the numbers of bacteria present in used sawdust or in wall scrapings point to the danger of greatly increased contamination of the meat resulting from accidental contact with such sources. The increased counts after cutting are due to the handling in the cutting room involving various cutting, sawing, trimming, and handling operations carried out during a period of approximately one-half hour.

TABLE II  
BACTERIAL COUNTS—VARIOUS SOURCES OF CONTAMINATION

Source	Agar plate count			
	0% NaCl	5% NaCl	10% NaCl	15% NaCl
Fresh sawdust, per gram	7,000	9,000	1,000	1,000
Used sawdust, per gram	216,000,000	190,000,000	73,000,000	750,000
Bloody sawdust, per gram	14,400,000,000	24,640,000,000	8,800,000,000	700,000
Wall scrapings, per gram	67,500,000	47,500,000	46,200,000	38,700,000
Exposed plates <sup>1</sup> —floor	180	125	15	5
Exposed plates—raised	90	33	23	15

<sup>1</sup> Area, 60 sq. cm.; exposed to air 15 min.

### Classification of Organisms

Examination of the plates of nutrient agar and of salt agar showed variations in the bacterial types developing. Special attention was given the "after cutting" series in an estimation of the relative incidence of different types. From plates or sectors of plates all colonies were picked and transferred to agar slants for further study. The percentage distribution of the various morphological types is illustrated in Fig. 1, which shows the changing proportions of the groups as they vary with the salt content of the medium.

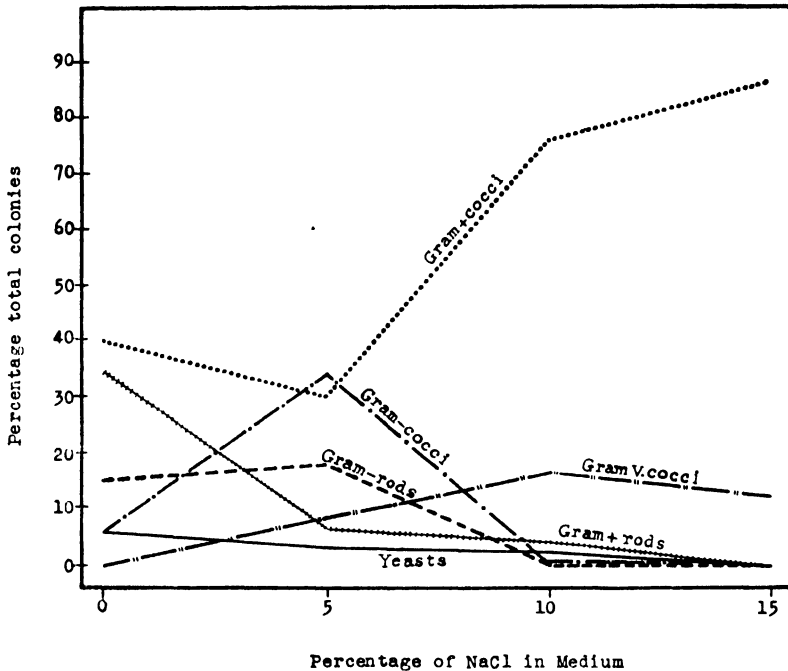


FIG. 1. Distribution of various morphological types on plates of different salt concentration, representing contamination of sides after cutting.

For more detailed study of the hundreds of colonies isolated it was necessary to concentrate on one isolation group. It was felt that the organisms on 5% salt agar plates would be most representative of those comprising initial contamination of meat to be pickled. This medium showed a well-assorted bacterial flora, with all morphological groups well represented, and gave comparatively high total counts. Facultative halophiles, which might be important in pickle, should be well represented on 5% salt agar, to the exclusion of certain non-halophiles. Finally, as 5% salt is a favourable concentration for isolating types occurring in bacon slime, it was felt that this concentration would be most likely to include organisms able to cause spoilage. Accordingly, 100 cultures from the 5% salt agar plates of the "after cutting" series, together with a group of 185 cultures from plates of the same medium prepared from sawdust, air, wall scrapings, and freshly slaughtered hogs,

were studied microscopically and culturally with the object of eliminating similar strains and reducing the number to those that could be regarded as different type species. For this differentiation, reliance was placed on Gram staining, nitrate reduction, gelatin liquefaction, lipolysis, litmus milk reaction, and fermentation of dextrose. All test media contained 5% sodium chloride.

The relative abundance of the morphological groups represented in the 285 cultures isolated from 5% salt agar plates is shown in Fig. 2, organisms from the "after cutting" series being compared with those from various sources before cutting. Micrococci, Gram positive and Gram negative, were found to comprise the most abundant groups, followed by Gram negative rods,

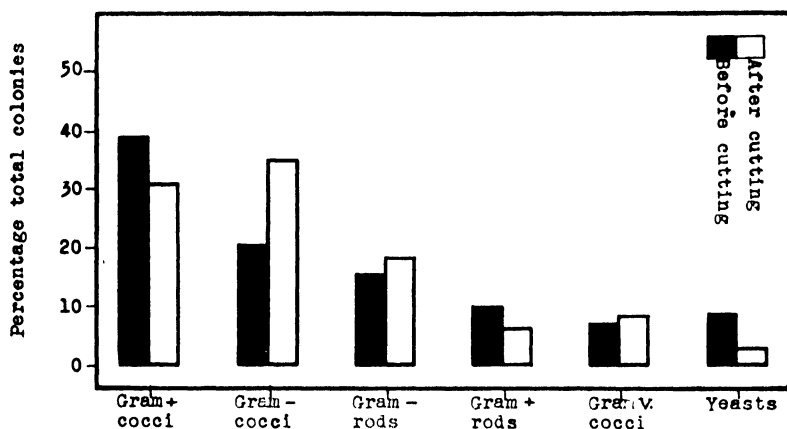


FIG. 2. Relative incidence of different groups of organisms from various sources before cutting, and from the "after cutting" series.

many of which were highly pleomorphic forms. A numerically important group, the classification of which presented some difficulty, was included as Gram negative cocci. These organisms were characteristic oval forms which might also be regarded as coccoid rods. As a group these showed relatively pronounced lipolytic action and were similar to organisms found by Landerkin (3) to be the most abundant types appearing on 5% salt agar plates from certain samples of slime on cured Wiltshire sides.

As a result of the comparative study of 100 cultures from the "after cutting" series, 28 bacterial cultures and one yeast were regarded as different types. Examination of the group of 185 cultures revealed 11 additional bacterial types. Forty cultures therefore were regarded as types representative of the 285 original isolations. Further study was made of the cultural characteristics of these organisms, details of which need not be given here. However, a summary of the 39 bacterial types representing pre-curing contamination is given in Table III, the organisms being grouped on the basis of morphology and more important physiological properties.

TABLE III  
SUMMARY OF BACTERIAL TYPES REPRESENTING CONTAMINATION PRIOR TO CURE

	No. of different types	Chromogenic	Motile	Spores	Gelatin liquefaction	Nitrate reduction	Lipolysis	H <sub>2</sub> S production	Acid in		
									Dextrose	Sucrose	Lactose
Micrococci, Gram pos.	10	5	0	0	5	6	6	4	8	7	4
Micrococci, Gram neg.	6	0	0	0	0	4	5	2	0	0	0
Micrococci, Gram. var.	4	0	0	0	1	2	1	1	4	4	2
Rods, Gram. pos.	9	2	0	1	4	4	1	1	2	2	0
Rods, Gram neg.	10	2	4	0	3	6	2	3	3	2	0
Total	39	9	4	1	13	22	15	11	17	15	6

### Relation of Organisms to Salt

The effect of salt on micro-organisms has been given much attention and there has accumulated, in consequence, a voluminous literature which need not be reviewed here. It is known that organisms from a salt-free environment can tolerate salt to varying degrees, with evidence of some stimulation by lower, and eventual suppression by higher concentrations. The degree of tolerance to salt is further modified by other environmental factors such as temperature, aeration, pH, organic nutrients, amount of inoculum, age of culture, and microbial association. It is further apparent that certain types, such as micrococci, from salt-free sources are able to withstand higher salt concentrations than others. From salt environment, on the other hand, both from natural and "industrial" sources, organisms have been found that prefer or require various concentrations of salt, some thriving best in an environment approaching saturation. Among such organisms micrococci are less predominant. That a considerable degree of adjustment to salt may occur seems well established, though there is still difference of opinion as to the suitability of, and distinction between, such terms as "obligate halophile", "facultative halophile", "salt tolerant", and "salt resistant".

Since organisms contaminating Wiltshire sides prior to curing are subjected to the influence of a salt pickle approximating saturation, it was considered a matter of practical interest to study the relation of the microbial types found, in various concentrations of sodium chloride. Information on this point should permit a better estimate of the importance of pre-curing contamination and its possible relation to later defects of the cured product. Tests were therefore made of the "salt tolerance" and "salt resistance"\* of 40 microbial types representing contamination, to which were added, for comparison, seven other organisms from various sources.

\* For the purpose of this paper, "salt tolerance" refers to ability to grow, and "salt resistance" ability to remain viable, in an environment of definite salt concentration.

## METHODS

*Salt tolerance.* From eight-day cultures of the organisms on 5% salt agar, faintly turbid suspensions were made in 5% sodium chloride solution, and one loopful of the suspension was used for inoculation. Heavy inocula were avoided, in view of the findings of certain workers (9, 10), who showed that mass effect caused by the addition of large inocula may increase salt tolerance, and thus tend to neutralize any inhibitive effects. Transfers were made to a series of slants of nutrient agar containing respectively 0, 5, 10, 15, 20, and 25% sodium chloride, in addition to 0.1% potassium nitrate. Transfers were also made by loop stab to a similar series of semi-solid media of the same composition except that 0.08% agar was used. Although the amount of agar used was so small that the medium did not appear to differ from straight broth, tests showed that when growth occurred it appeared as a suspended localized growth, which showed to advantage compared to a general distribution in broth, and made the recognition of bacterial multiplication easier.

Inoculated tubes were incubated at room temperature for two weeks in the case of 0 and 5% sodium chloride media, and for three weeks with the higher concentrations. Tightly rolled plugs allowed but a minimum of evaporation. The tubes were examined for growth and tested for nitrites. Organisms showing growth were adjudged to have tolerated the salt concentration in question.

*Salt resistance.* By means of a measured loop, equivalent amounts of 10-day cultures were transferred to 10 ml. of 5% sodium chloride solution. This was agitated and an endeavour made to establish as uniform turbidity as possible. One-tenth ml. of suspension of each culture was transferred to two tubes of 5% sodium chloride broth, to one tube each of 10, 20, and 30% sodium chloride broth and to one tube of sterile pickle (29.75% sodium chloride). The salt broths contained, in addition to sodium chloride, 0.1% potassium nitrate, 0.15% beef extract, and 0.25% peptone. The pickle was a filtered curing brine, as used for Wiltshire sides. All tubes contained 10-ml. quantities.

From one of the duplicate 5% sodium chloride tubes, plates were poured with 5% salt agar and incubated at room temperature for two weeks. The counts gave the original count per ml. at time of inoculation. At the end of 5 and 10 days' incubation at room temperature, times that correspond approximately with the duration of cure at various plants, estimates were made of the numbers of organisms in each inoculated tube by plating on 5% salt agar. Since larger amounts taken from the tubes of the higher salt concentration would affect the sodium chloride concentration and hence the counts of the agar plates, transfers were made by loop. Calibration for each sodium chloride broth was necessary owing to the effect of specific gravity on the volumes delivered by the same loop. Preliminary tests were made in weighing loopfuls of each salt concentration. Repeated weighings showed that out of 120 loopfuls there was rarely more than one or two loopfuls difference each

time. In plating, the loopful was mixed with sterile 5% salt solution in the plate and gently tapped to rid the loop of all its contents. This followed analogous procedure in standardizing. Dilution, if necessary before plating, was made in sodium chloride solution. Plates were incubated at room temperature for two weeks before counting.

## RESULTS

Results from the salt tolerance tests showed good agreement between the agar and semi-solid inoculations. The semi-solid medium showed if anything slightly better growth, and in Table IV are summarized the results from this medium, arranged for the morphological groups. Gram negative micrococci show a pronounced decline in salt tolerance between sodium chloride concentrations of 10 and 15%, whereas Gram positive micrococci display a greater salt tolerance. There was little difference between the Gram positive and Gram negative rods, considered as groups. The tests show that many organisms isolated on 5% salt agar from an apparently salt-free environment

TABLE IV  
SALT TOLERANCE (CAPACITY FOR GROWTH) OF MICRO-ORGANISMS REPRESENTING  
CONTAMINATION PRIOR TO PICKLING

Morphological group	No. of types in group	Number showing growth in semi-solid medium					
		0% NaCl	5% NaCl	10% NaCl	15% NaCl	20% NaCl	25% NaCl
Contaminants from plant							
Micrococci, Gram pos.	10	10	10	10	9	3	1
Micrococci, Gram neg.	6	6	6	6	1	1	0
Micrococci, Gram. var.	4	4	4	3	3	0	0
Rods, Gram pos.	9	9	9	9	5	2	1
Rods, Gram neg.	10	9	10	9	5	2	0
Yeasts	1	1	1	1	0	0	0
Total	40	39	40	38	23	8	2
Control Cultures							
<i>Staph. aureus</i>		+	+	+	+	-	-
<i>Esch. coli</i>		+	+	-	-	-	-
<i>Achromobacter</i> sp. from slime (No. 44)		+	+	+	+	+	-
<i>Micrococcus</i> sp. from pickle (No. 42)		-	+	+	+	+	-
<i>Achromobacter</i> sp. from pickle (No. 45)		-	-	+	+	+	+
<i>Micrococcus</i> sp. (No. 46)		+	+	+	+	+	-
<i>Micrococcus</i> sp. (No. 47)		+	+	+	+	+	-

in a packing plant can tolerate relatively high sodium chloride concentrations, more than one-half being able to grow on media ranging from 0 to 15% sodium chloride, and one-fifth up to 20%. A concentration of 25% sodium chloride, however, appears to be definitely inhibitive to organisms isolated and carried on a 5% sodium chloride medium. That some adjustment to high salt environment of pickle is possible, however, is suggested by the range of adaptability shown by many types.

Since part of the value of a pickle flora depends upon the presence of nitrate-reducing bacteria, cultures were tested for nitrite. Of those shown in Table IV, 26 species reduced nitrate to nitrite in 5% sodium chloride medium. In addition to the original series with light inoculum, a special series was prepared in which heavy inoculations were made from the 26 nitrate-reducing organisms. Results are shown in Table V. It is of interest to note from the

TABLE V  
COMPARISON OF GROWTH AND NITRATE REDUCTION AT DIFFERENT SALT CONCENTRATIONS

26 species reducing NO <sub>3</sub> at 5% NaCl concentration	NaCl concentration					
	0%	5%	10%	15%	20%	25%
<i>Light inoculum</i>						
Growth	24	26	26	15	8	3
Nitrate reduction	24	26	24	11	4	1
<i>Heavy inoculum</i> <sup>1</sup>						
Nitrate reduction	24	26	24	12	6	1

<sup>1</sup> Owing to heavy inoculum, growth could not be determined in this series.

"light inoculum" series, that growth up to a concentration of 5% sodium chloride was accompanied by nitrate reduction. As the sodium chloride concentration of the medium increased, however, nitrate reduction occurred with a progressively smaller proportion of the species showing growth, until at 25% sodium chloride concentration the only organism showing reduction was one of the "controls", a halophile isolated from curing pickle. Mass inoculation produced relatively little effect on nitrate reduction. The findings are of interest in showing that nitrate reduction is interfered with before growth is suppressed, and suggest that reduction of nitrate in curing pickle is a function to be ascribed to the true halophiles rather than to the pre-curing contaminants.

In the salt resistance experiments, in which 47 cultures were placed in five salt environments and initial, 5-day, and 10-day (and in one set, 30-day) counts recorded, 564 analyses were made. In portraying this mass of data, ranging from very high to very low counts, it was felt that the findings could best be summarized in, (a) a graph showing the trend of groups according to their behaviour in salt, and (b) a table expressing the reaction according to morphological types. These are shown respectively in Fig. 3 and Table VI.

According to the action of the organisms in salt solution, five distinct trends were shown by the counts (Fig. 3).

*Groups 1 and 2.* Closely related groups, comprising nine species of Gram positive micrococci, four Gram positive and two Gram negative rods, which maintained their numbers relatively well in 20% and 30% sodium chloride and in pickle. Group 2 differed from Group 1 by showing higher counts in pickle than at the start, the only group to show this.

TABLE VI  
SALT RESISTANCE (SURVIVAL) OF MICRO-ORGANISMS REPRESENTING CONTAMINATION PRIOR TO PICKLING

Morphological group	No. of types group in	Days	5% NaCl			10% NaCl			20% NaCl			30% NaCl			Pickle (29.75% NaCl)		
			Inc.	Stat.	Dec.	Inc.	Stat.	Dec.	Inc.	Stat.	Dec.	Inc.	Stat.	Dec.	Inc.	Stat.	Dec.
Micrococci, Gram pos.	10	5	10			9		1		4	5		4	2		9	1
		10	10			8		1		3	4		2	3		8	2
		10	10														
Micrococci, Gram neg.	6	5	6			4				2	3			4		1	4
		10	6			5		1			4			3		1	5
		10	6														
Micrococci, Gram var.	4	5	4			4					1			1		2	1
		10	4			2		1		1	1			1		1	1
		10	4														
Rods, Gram pos.	9	5	9			6		2			6			3		5	4
		10	9			5		4			4			2		4	5
		10	9														
Rods, Gram neg.	10	5	10			5		1		2	4			5		3	3
		10	10			6		1		3	3		1	2		5	4
		10	10														
Yeast	1	5	1					1									1
		10															1
		10															
Total	40	5	40			28		4		6	2		2	12		6	17
		10	39			1		1		7	6		1	11		5	13
		10	39											16		2	22
<i>Staph. aureus</i>		5	x			x					x			x			x
		10	x			x								x			x
		10	x			x											
<i>Esch. coli</i>		5	x			x					x			x			x
		10	x			x											
		10	x			x											
<i>Achromobacter</i> sp. (No. 44)		5	x			x											
		10	x			x											
		10	x			x											
<i>Micrococcus</i> sp. (No. 42)		5	x			x					x			x			
		10	x			x											
		10	x			x											
<i>Micrococcus</i> sp. (No. 46)		5	x			x											
		10	x			x											
		10	x			x											
<i>Micrococcus</i> sp. (No. 47)		5	x			x											
		10	x			x											
		10	x			x											

Inc. = Increase over initial counts of 100% or more. Dec. = Decrease in count of 50% or more. Stat. = Within the above limits for increase or decrease. Inh. = Inhibition. No growth on plates.

**Group 3.** Four Gram positive, three Gram negative, and one Gram variable species of micrococci, two Gram positive and one Gram negative rods, which declined in numbers from 5 to 30% sodium chloride solution, but maintained numbers up to 10 days in pickle, though declining after one month.

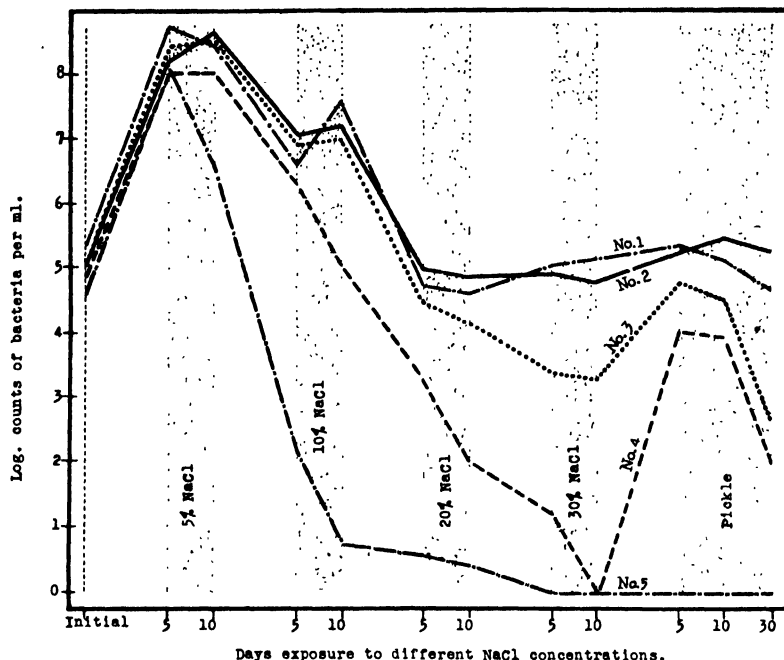


FIG. 3. Survival of 46 types of micro-organisms grouped according to their resistance in salt broths of different sodium chloride concentrations and in pickle.

**Group 4.** Fifteen cultures, representing all morphological types with rod forms predominating. This group showed a noticeable decline in survivors from 5 to 30% sodium chloride, at which latter concentration no species appeared to survive after 10 days. In pickle of approximately the same concentration however, there was a good measure of survival during the same time.

**Group 5.** Four Gram negative rods, together with the one yeast type. These organisms were the most susceptible, declining rapidly in sodium chloride concentrations above 5% and showing no evidence of survival in either 30% sodium chloride or in pickle.

Reactions according to morphological types are given in Table VI, and summarized according to the values "increase", "decrease," "static", or "inhibition", corresponding to standards\* set on the basis of the plate counts made after 5 and 10 days respectively. From the table may be seen the relatively greater resistance of Gram positive micrococci and the comparative

\* For definition of terms see footnote to Table VI.

susceptibility of Gram negative rods towards higher salt concentrations. The most striking finding is the much greater resistance displayed in pickle than in salt broths of similar sodium chloride content. Whereas 15 species were completely inhibited after 5 days, and 22 species after 10 days in 30% sodium chloride broth, but 5 and 6 species respectively were inhibited in pickle of almost identical sodium chloride concentration. On the other hand, whereas only 5 species showed static counts at the end of 10 days in 30% sodium chloride solution, 16 species showed static or increased counts in pickle, and these comprised 65% of the total number of organisms representing original contamination. It appears that curing pickle possesses substances that neutralize or mask the toxic or inhibitive action of high salt concentration, despite the fact that it was sterilized at 15 lb. for 30 min., filtered through paper to remove precipitate, and re-sterilized. Comparing the pickle totals with those for the sodium chloride broths, the inhibitive effect of the former is less than that of 20% sodium chloride broth, approximating that of a solution of one-half its actual salt strength. That the decreases and inhibitions in the salt broths were not owing to lack of nutrient is borne out by the high increases in the lower salt concentrations. The effect noted in pickle is "preservative" rather than "stimulative", as seen from the totals in the "static" column (Table VI). This is further supported by tests of plating media prepared for pickle, which showed no advantage over other media of similar sodium chloride strength.

To examine the possibility that organisms introduced to an environment of 30% sodium chloride might adjust themselves to better growth on higher concentration of salt agar than on the 5% sodium chloride medium used, all cultures that showed inhibition in 30% sodium chloride broth were subjected to a control test in which plating was made on 10 and 15% sodium chloride agar as well as on the regular 5% sodium chloride medium. In no case did cultures that showed inhibition, as judged by plating on 5% agar, show growth on the 10 or on the 15% sodium chloride agars, permitting the deduction that with the 23 species studied the action of 30% salt involved a toxic effect rather than an adjustment to higher salt concentrations.

It appears that many types of organisms, constituting original contamination of Wiltshire sides, are able to survive the pickling process. Although the findings do not point to any pronounced activity of such organisms in pickle, either beneficial or deleterious, their ability to survive opens the possibility of their becoming active on sides after pickling, and contributing to storage defects. The findings therefore point unmistakably to the importance of maintaining packing plants hygienically clean in an effort to reduce this initial contamination to a minimum.

### Acknowledgment

The authors wish to express their appreciation of the assistance received from Canada Packers Limited, and especially the co-operation of Mr. W. J. Hodder in the furnishing of samples and in providing facilities for plant tests.

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# Canadian Journal of Research

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## HYDROIDS OF THE WESTERN CANADIAN ARCTIC REGION, 1935-1937<sup>1</sup>

BY C. MCLEAN FRASER<sup>2</sup>

The hydroids here reported upon were obtained by Sergeant H. A. Larsen of the Royal Canadian Mounted Police vessel *St. Roch* in July and August, 1936 and 1937\*. While the vessel was stuck in the ice, Sergeant Larsen made a dredge which was lowered to the bottom and pulled along by the drift of the ice. The material was preserved and later deposited with the Pacific Biological Station, where it was sorted by Dr. Josephine F. L. Hart.

Although this material was not very extensive, it proved to be as interesting as any yet obtained from the Canadian Arctic. Of the five species in the collection, two appear to be new and one of these belongs to a genus quite different to anything previously described.

In Dease Strait, 68° 58' N, 106° 20' W, in 40 fathoms, the four species *Calycella syringa* (Linnaeus), *Thuiaria similis* (Clark), *Thuiaria tenera* (Sars), and a new species of *Bonneviella*, were obtained, and off Cape Bexley, Dolphin and Union Straits, 68° 59' N, 115° 40' W, in 9 fathoms, the new genus *Meganema* with the new species *M. claviformis*.

### Description of Genus and Species

#### Genus *Bonneviella*

*Bonneviella gracilis* new species. Fig. 1, a, b, c.

**Trophosome.** Zooids growing singly from a slender stolon that winds about on colonies of *Thuiaria similis*. Pedicels slender, varying in length, the longest 2.5 mm., without annulations except the one at the base of the hydrotheca. Hydrotheca slender with length varying from 2 to 2.5 times the width, greatest

<sup>1</sup> Manuscript received September 26, 1938.

Contribution from the Department of Zoology, The University of British Columbia, Vancouver, Canada.

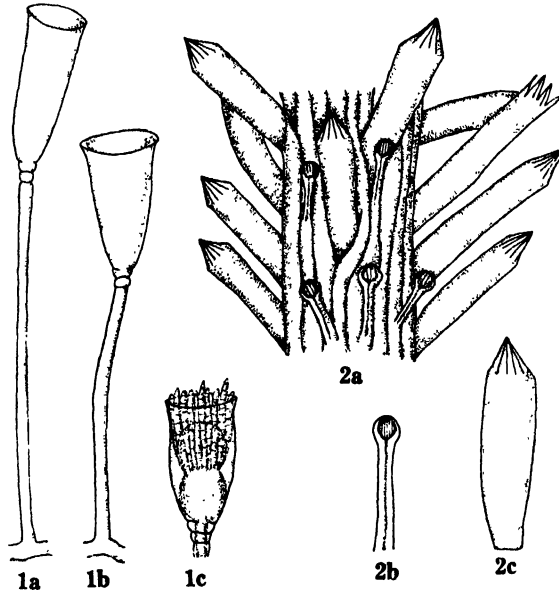
<sup>2</sup> Professor of Zoology, The University of British Columbia.

\* During the time that the Royal Canadian Mounted Police vessel "St. Roch" was on patrol duty in the Canadian Arctic from 1935 to 1937, Sergeant H. A. Larsen, in charge, took a number of hydrographic observations with instruments supplied by the Pacific Biological Station, and also made some collections of biological material. The data and collections have been deposited with the Station, where they are being examined in part, while certain groups are being submitted to specialists for study. A series of reports will be prepared for publication.

To the Royal Canadian Mounted Police and to Sergeant Larsen, the Fisheries Research Board of Canada is greatly indebted for these collections, which will contribute to the biology and hydrography of Arctic waters.

W. A. Clemens, Director, Pacific Biological Station, Nanaimo, B.C.

length 1.0 mm., tapering very gradually from base to margin and sometimes slightly urceolate. Margin slightly flaring, entire. Hydranth with about 20 tentacles. These have rings of nematocysts almost like those on the tentacles of *Gonionemus*, although they are not so pronounced.



FIGS. 1 AND 2. Magnification  $\times 20$ . FIG. 1. *Bonneviella gracilis*. a and b, hydrotheca and pedicels. c, hydrotheca with hydranth. FIG. 2. *Meganema claviformis*. a, portion of fascicled stem to show hydrothecae and tentacular organs; b, a single tentacular organ; c, a single hydrotheca.

NOTE: Apart from its generic characters this species bears little resemblance to other species of the genus. The relatively small size of the hydrotheca, which is campanulate rather than tubular, with an entire, even, flaring margin, sets it distinctly apart from all other species.

Gonosome. Not observed.

#### FAMILY CAMPANULINIDAE

##### Genus *Meganema* new genus

*Trophosome*. Colony fascicled, with sessile hydrothecae of the *Campanulina* type, arising from the central or axial tubes. These axial tubes are covered by more slender peripheral tubes from which extend long stalked nematophores or tentacular organs.

Gonosome. Unknown.

*Meganema claviformis* new species. Fig 2, a, b, c.

*Trophosome*. Colony stout, of much the same size throughout, up to 4 cm. in length, without branches, attached to sponges, barnacle shells, etc. The

peripheral tubes are about half the diameter of the axial tubes. Hydrothecae irregularly arranged, coming out from the axial tubes between the peripheral tubes; they are almost regularly tubular, varying more in length (maximum 1.25 mm.) than in width (0.3 mm.). There is no definite margin at the base of the opercular segments, which are ten in number, narrowing uniformly to a point from all sides as in *Campanulina*. The nematophores, or tentacular organs, are numerous, presenting a striking feature of the species; they come off the peripheral tubes, with long pedicels, 0.6 to 0.8 mm., less than 0.1 mm. in width. The terminal bulb is somewhat larger than, but not twice as large as, the diameter of the pedicel.

*Gonosome*. Not observed.

### Acknowledgments

The author wishes to express his thanks to Dr. W. A. Clemens and the Fisheries Research Board for the opportunity of examining the hydroids, and to Miss Ursula Dale for the drawings used in the illustration.

## CUMACEA AND DECAPODA OF THE WESTERN CANADIAN ARCTIC REGION, 1936-1937<sup>1</sup>

BY JOSEPHINE F. L. HART<sup>2</sup>

### Cumacea

Sixteen species of Cumacea were dredged at five localities southwest of Victoria Island, in the western Canadian Arctic region in 1936-37, by Sergeant H. A. Larsen, master of the R.C.M.P. schooner "St. Roch". Although the Canadian Arctic Expedition investigated part of the same area, only three species of Cumacea were obtained (1). Two of these were taken again and the third, *Diastylis rathkii* Kroyer, has subsequently (7) been divided into a number of subspecies and *D. oxyrhyncha*, of which young individuals occur in the present collection. The other species found seem to be new records for the area and are of interest as a link between the fauna of the North Atlantic and of Alaska, where rather extensive studies on the Cumacea have been made. On the whole, the species are North Atlantic or Arctic forms rather than Alaskan. The extension of range is therefore westward.

Details of distribution have been given by recent authors and thus only those localities are listed here that are of interest because of their proximity to the region concerned. The difficulty of positive identification in some instances has been increased by the predominance of immature forms in the collection.

Two species are of interest because of their comparative rarity: *Brachydiastylis nimia* Hansen would appear to have been taken only once before and then off East Greenland and *Cumella carinata* Hansen four times previously, the locality closest to the present one being at Ellesmere Land (79° 30' N, 106° W). None of the other species is especially rare off Greenland or in Europe, and further investigation will probably obliterate the gaps between.

### FAMILY LEUCONIDAE

#### *Leucon nasicooides* Lilljeborg

Locality. Dease Strait, 68° 58' N, 106° 25' W, 40 fms., July 25, 1937, 1 ♀ 4 mm.

Western limit. Gulf of St. Lawrence.

#### *Leucon fulvus* Sars

Locality. Dease Strait, 69° N, 106° 25' W, 45 fms., August 7, 1936, 1 ♂ mature 5 mm., 4 ♀ 4 mm.

Dease Strait, 68° 58' N, 106° 25' W, 40 fms., July 25, 1937, 4 ♀ 4 mm.

Bernhard Harbour, 68° 50' N, 114° 55' W, 5 fms., Aug. 22, 1936, 1 ♂ mature 5 mm.

Western limit. Davis Strait, 66° 35' N, 56° 38' W.

<sup>1</sup> Manuscript received September 26, 1938.

<sup>2</sup> Contribution from the Pacific Biological Station, Nanaimo, B.C.

<sup>3</sup> Temporary Assistant, Pacific Biological Station, Nanaimo, B.C.

Remarks. The male from Dease Strait has two teeth at the apex of the frontal lobe. Both males have three subequal lanceolate setae and one short seta on the third leg, agreeing with Hansen's (2) but not with Sars' (5) descriptions.

*Leucon pallidus* Sars

Locality. Dease Strait, 69° N, 106° 25' W, 45 fms., Aug. 7, 1936, 1 ♀ 3.5 mm.

Dease Strait, 68° 58' N, 106° 25' W, 40 fms., July 25, 1937, 3 ♂ 4 mm.

Western limit. Davis Strait, 66° 35' N, 56° 38' W.

*Leucon acutirostris* Sars

Locality. Dease Strait, 69° N, 106° 25' W, 45 fms., Aug. 7, 1936, 5 ♂ 4 mm., 1 ♀ 3 mm., 1 broken.

Dease Strait, 68° 58' N, 106° 25' W, 40 fms., July 25, 1937, 1 ♂ 4 mm.

Western limit. Davis Strait, 66° 35' N, 56° 38' W.

*Leucon nasica* (Kroyer)

Locality. Dease Strait, 69° N, 106° 25' W, 45 fms., Aug. 7, 1936, 4 ♀ ovig. 8.5 mm., 20 ♂ and 116 immature.

Dease Strait, 68° 58' N, 106° 25' W, 40 fms., July 25, 1937, 1 ♀ 8 mm.

Bernhard Harbour, 68° 50' N, 114° 55' W, 5 fms., Aug. 22, 1936, parts of about 10.

Western limit. Beachy Island, Labrador, and southern Alaska.

FAMILY CAMPYLASPIDAE

*Campylaspis rubicunda* (Lilljeborg)

Locality. Dease Strait, 69° N, 106° 25' W, 45 fms., Aug. 7, 1936, 4 ♀ 4.5 mm. and 3 mm., 3 ♂ 3 mm. and 4.5 mm.

Bernhard Harbour, 68° 50' N, 114° 55' W, 5 fms., 1 ♂ lacking abdomen.

Western limit. West Greenland.

Remarks. There are no definite teeth on the first and second pedigerous segments, and the propods are stouter than indicated by Sars' figures, but these are probably due to the immature state of the specimens, as the colour and the four spines on the dactylus of the second maxilliped are like *C. rubicunda*. Hansen (2) considers the latter character quite distinctive.

*Campylaspis costata* Sars

Locality. Dease Strait, 69° N, 106° 25' W, 45 fms., Aug. 7, 1936, 1 ♂ 4.5 mm.

Dease Strait, 68° 58' N, 106° 25' W, 40 fms., July 25, 1937, 1 ♂ 3 mm.

Western limit. Irish Sea.

Remarks. The tubercles are more prominent than usual, the lateral carina being joined dorsally by a series of tubercles rather than by carina. Elongated

red-brown chromatophores outline the carina and tubercles, the margin of the carapace, the abdomen, and the appendages.

FAMILY NANNASTACIDAE

*Cumella carinata* Hansen

Locality. Dease Strait, 68° 58' N, 106° 25' W, 40 fms., July 25, 1937, 2 specimens 3 mm.

Western limit. Off Ellesmere Land, 79° 30' N, 106° W.

Remarks. Both specimens are encrusted with a *Vorticella*-like ciliate.

FAMILY DIASTYLIDAE

*Diastylis spinulosa* Heller

Locality. Dease Strait, 69° N, 106° 25' W, 45 fms., Aug. 7, 1936, 1 ♀ ovig. 20 mm. uropods broken, 1 specimen 10 mm., 2 specimens 6 mm. (lack last pair of legs).

Dease Strait, 68° 58' N, 106° 25' W, 40 fms., July 25, 1937, 1 specimen 6 mm. Dolphin and Union Strait, 69° 5' N, 115° 45' W, 60 fms., Aug., 1937, 1 specimen 6 mm.

Western limit. Dolphin and Union Strait, 68° 50' N, 115° W.

Remarks. All specimens have more and sharper spines than are described from European specimens. Calman (1) noted more numerous spines.

*Diastylis goodsiri* Bell

Locality. Dease Strait, 69° N, 106° 25' W, 45 fms., Aug. 7, 1936, 2 specimens 8 mm. (no fifth legs), posterior part of slough of ♂, 13 mm.

Dease Strait, 68° 58' N, 106° 25' W, 40 fms., July 25, 1937, 1 specimen 5 mm. (no fifth legs).

Western limit. Dolphin and Union Strait, 68° 50' N, 115° W.

Remarks. The small specimens have the carapace covered with fine spines and hairs.

*Diastylis edwardsi* Kroyer

Locality. Dease Strait, 69° N, 106° 25' W, 45 fms., Aug. 7, 1936, 1 ♂ 6 mm., 2 specimens 4 mm. (no fifth legs).

Dease Strait, 68° 58' N, 106° 25' W, 40 fms., July 25, 1937, 1 ♂ 8 mm., 3 specimens 4.5 mm. and 3 mm. (no fifth legs).

Western limit. Baffin Land, 72° 38' N, 77° 10' W.

Remarks. One specimen has the point on the fifth pedigerous segment more pronounced than is usual.

*Diastylis oxyrhyncha* Zimmer

Locality. Dease Strait, 69° N, 106° 25' W, 45 fms., Aug. 7, 1936, 1 abdomen of ♂ whose total length would be about 17 mm., 2 ♀ 13 mm., 5 specimens

10 mm., 3 specimens 8 mm., 12 specimens 5 mm. (no fifth legs) 23 specimens 3 mm.

Bernhard Harbour, 68° 50' N, 114° 55' W, 5 fms., Aug. 22, 1936, 2 damaged specimens, 5 and 3 mm.

Western limit. Baffin Bay, 72° 4' N, 59° 50' W.

Remarks. Although all the specimens are immature, the larger are so like Zimmer's (7) description and figure, that there seems to be little doubt as to their identity. The smaller specimens are definitely of the *D. rathkii* group and probably of the same species. Calman (1) records *D. rathkii* from Bathurst Inlet, 67° 35' N, 108° 40' W, and as *D. oxyrhyncha* was included then with this species, it is possible that both records are of the same species.

*Leptostylis villosa* Sars

Locality. Dease Strait, 69° N, 106° 25' W, 45 fms., Aug. 7, 1936, 9 ♀ ovig. 4 mm., 100 ♀ 3.5–4 mm., 50 ♂ 4 mm.

Dease Strait, 68° 58' N, 106° 25' W, 40 fms., July 25, 1937, 7 ♂ 3.5 mm., 13 ♀ 3.5 mm.

Bernhard Harbour, 68° 50' N, 114° 55' W, 5 fms., Aug. 22, 1936, broken 1 ♂ and 7 ♀.

Western limit. Davis Strait, 66° 35' N, 56° 38' W.

*Leptostylis ampullacea* (Lilljeborg)

Locality. Dease Strait, 69° N, 106° 25' W., 45 fms, Aug. 7, 1936, 1 ♂ 5 mm., 1 ♀ 4.5 mm., 1 specimen 4 mm.

Dease Strait, 68° 58' N, 106° 25' W, 40 fms., July 25, 1937, 1 ♀ 4.5 mm., 1 specimen 2 mm. (no fifth legs).

Western limit. Gulf of Maine.

*Brachydiastylis resima* (Kroyer)

Locality. Cape Krusenstern, Coronation Gulf, 10 fms., Aug. 1937, 1 ♀ 5 mm. (damaged).

Western limit. Baffin Bay, 72° 38' N., 77° 10' W.

Remarks. As pointed out by Hansen (2), there is a spinous process on the exterior margin of the second joint of the third pair of legs and the inner terminal seta of the exopod of the uropod is considerably longer than Sars' (5) figure.

*Brachydiastylis nimia* Hansen

Locality. Dease Strait, 69° N, 106° 25' W, 45 fms., Aug. 7, 1936, 2 ♀ ovig. 3.5 mm., 4 ♀ 3–3.5 mm., 1 specimen 3 mm.

Western limit. East Greenland, north of Stewart Land, 70° 30' N.

Remarks. One female is as described by Hansen (2), except that the teeth on the lateral margin of the carapace are somewhat more recurved than his figure would indicate. The other mature female and one of the immature,

has in addition, three square teeth, almost mushroom-shaped, between the large antero-lateral tooth and the base of the pseudorostral lobes. The remainder have four or five similar teeth on each side of the anterior part of the carapace.

### Decapoda

The decapod collection from the vicinity of Victoria Island is a small one, comprising only seven species of shrimps. Six of these were obtained by the Canadian Arctic Expedition (4) from much the same area, and the seventh, *Argis dentata* Rathbun, has been taken in the Bering Sea (3) and off Baffin Land and in Hudson Bay (6).

#### FAMILY HIPPOLYTIDAE

##### *Spirontocaris groenlandica* (J. C. Fabricius)

Locality. Cape Bexley, Dolphin and Union Strait, 9 fms., ♂ 43 mm., ♀ 39 mm., rostrum broken.

##### *Spirontocaris spina* (Sowerby)

Locality. Cape Bexley, Dolphin and Union Strait, 9 fms., 2 ♀, 47 and 20 mm.

##### *Spirontocaris phippsii* (Kroyer)

Locality. Dease Strait, 68° 58' N, 106° 20' W, 40 fms., July 25, 1937, ♀ 37 mm.

##### *Spirontocaris polaris* (Sabine).

Locality. Cape Bexley, Dolphin and Union Strait, 9 fms., ♀ 35 mm. with isopod parasitic in right gill chamber.

Dease Strait, 68° 58' N, 106° 20' W, 40 fms., July 25, 1937, ♂ 53 mm. (no dorsal teeth on rostrum), ♀ 61 mm.

##### *Spirontocaris fabricii* (Kroyer)

Locality. Cape Bexley, Dolphin and Union Strait, 9 fms., ♂ 45 mm., ♀ 50 mm.

Cape Krusenstern Harbour, Coronation Gulf, 10 fms., Aug. 12, 1937, 5 ♂, 47, (2) 40, 36, and 24 mm. (rostrum broken).

#### FAMILY CRAGONIDAE

##### *Argis dentata* (Rathbun)

Locality. Cape Krusenstern Harbour, Coronation Gulf, 10 fms., Aug. 12, 1937, 12 mm.

Remarks. This immature specimen was kindly identified for the author by Dr. Belle A. Stevens, to whom grateful thanks are extended.

*Sabinea septemcarinata* Sabine

Locality. Cape Bexley, Dolphin and Union Strait, 9 fms., 1 damaged specimen about 25 mm.

Dease Strait, 69° N, 106° 25' W, 45 fms., Aug. 7, 1936, 2 ♀ 18 mm.

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# STUDIES ON THE BIONOMICS AND CONTROL OF THE BURSATE NEMATODES OF HORSES AND SHEEP

## VI. ON THE LETHAL EFFECTS OF SOME NITROGENOUS CHEMICALS ON THE FREE-LIVING STAGES OF SCLEROSTOMES<sup>1</sup>

BY I. W. PARNELL<sup>2</sup>

### Abstract

The effect of ten chemicals, containing a nitrogen radicle, on the free-living stages of *Sclerostomes* is discussed. Chloropicrin is the most lethal chemical yet tested; under the conditions of these tests, one part of chloropicrin will sterilize approximately 2,300 times its weight of fresh faeces, but its disadvantages weigh against its practicability for farm use. Aniline will sterilize about 525 times its weight of fresh faeces when undiluted and 800 or 900 times its weight as a very weak solution. Calcium cyanide, whose value is also limited by its danger, will sterilize 530 times its weight of fresh faeces. Pyridine is slightly more effective when applied undiluted or as a strong or medium strength solution; it will then sterilize about 400 times its weight of fresh faeces. Ammonium carbonate will sterilize approximately 50 times its weight of fresh faeces. Ammonium chloride, cupric nitrate, and ammonium nitrate will sterilize approximately 30, 25, and 21 times their own weight of fresh faeces respectively. They are, however, more effective if applied as medium strength solutions, and cupric nitrate in lesser amounts causes the death of many larvae after they have reached the third stage, although the chemical was added to the fresh faeces. Ammonium sulphide, as a 15% solution, will sterilize nearly 14 times its weight of fresh faeces. Saponin probably has no lethal value against *Sclerostomes* chemically, although physically it may affect them.

This paper reports on the lethal values against *Sclerostomes* in fresh faeces of some chemicals containing a nitrogen radicle, *viz.*, chloropicrin, aniline, calcium cyanide, pyridine, ammonium carbonate, ammonium chloride, cupric nitrate, ammonium nitrate, ammonium sulphide, saponin.

Previous papers (19, 21) have reported on urine and ammonia and on ten of the more common nitrogenous artificial fertilizers.

Of the chemicals discussed in this paper, ammonium nitrate is the only one which is commonly used as a nitrogenous fertilizer, but it is most frequently used as a constituent of a mixed fertilizer. However, chloropicrin, calcium cyanide, and pyridine are all reported to act as plant stimulants when used in smaller quantities than those necessary to check growth.

The technique used to obtain the data reported in this paper was that previously described (18, 22), except that a few of the cultures made with the larger quantities of chloropicrin and calcium cyanide (Series CXVIII, CXXXIV, and CVL) were not kept in the constant temperature room, owing to its lack of ventilation.

Some of the chemicals discussed in this paper have been tested for their effect on plant nematodes, but practically no work has been done with them in regard to the bursate nematodes. However, some of the results on plant

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nematodes and other pests are sufficiently comparable to be worth considering; especially is this true of chemicals that might be tried on grassland, where they might serve two or more purposes at the same time. It must be remembered that a nitrogenous chemical may make the interpretation of results on plant nematodes difficult because of the stimulation of the host plant by nitrogen.

The use of chloropicrin in the control of plant nematodes has been chiefly against the species that attack pineapples and bulbs. In pineapple fields (6, 7, 9) *Heterodera radicola* has been reduced by about 90% when chloropicrin has been used at the rate of 120 to 170 lb. per acre, especially when it has been applied in holes not more than 18 in. apart. When the land was covered with mulching paper its effectiveness was increased with the length of time the gas was retained. When the amount was increased to 350 lb., increased control resulted.

With holes 12 in. apart and no mulching paper, it has been shown (14) that 163 lb. per acre of chloropicrin was effective in controlling *H. marioni*; under similar conditions 1,000 lb. of calcium cyanamide was not effective. According to the author's technique, the ratio of effectiveness between chloropicrin and calcium cyanamide against *Sclerostomes* is almost 1 : 50 (20). *H. marioni* has been controlled (8) by 0.6 cc. and over of chloropicrin in covered pots holding 12 kg. of sandy soil. Chloropicrin has also the value of being lethal to wireworms (23), and although only slightly soluble in water, it is moderately effective when applied as a 1 : 3,000 solution, and more so when made into an emulsion with an equal quantity of fish oil and then mixed with water at 1 : 500 (24); in this form it has not yet been tried against *Sclerostomes*.

Calcium cyanide is frequently used against rodents and some of the larger insect pests. In pot trials against plant nematodes (13) it has been shown to be of value. However, against *H. schachtii* calcium cyanide was inferior to carbon disulphide (4) and drained creosote salts; carbon disulphide will be discussed in a subsequent paper, where it will be shown that against horse *Sclerostomes* the order of efficiency is reversed. Edwards (5) also found that 336 lb. per acre of calcium cyanide was ineffective against *Anguillulina dipsaci*. It may increase the yield of potatoes (10), and used at the rate of 750 to 2,000 lb. per acre (11), it substantially reduced nematode infection; on lightish loamy soil, with the other conditions favourable, 900 to 1,200 lb. increased the number of plants free from eelworm galls to about 90%.

Sodium cyanide, also because of the hydrocyanic acid gas which it liberates, especially when mixed with ammonium sulphate (2, 25), has been recommended for sterilizing small areas of soil. More cyanide is neutralized by a heavy than by a light soil (17). In excessive quantities it may retard the germination of seeds, although in small quantities it may be a stimulant. Potassium cyanide, which also liberates hydrocyanic acid, has been reported (1) to control *H. marioni* when used at the rate of 800 lb. per acre.

Pyridine and aniline do not appear to have been tested as lethal agents in soil, but they have both been tested against fly larvae in manure (3). Used

at the rate of about 80 lb. of solution per 10 cu. ft. of manure, pyridine, as a 1 : 100 and as a 1 : 500 aqueous solution, was reasonably effective, but was not as a 1 : 1,500 solution. Aniline at the same rate was also successful when used as a 1 : 400 or stronger aqueous solution, and as a 1 : 500 solution it caused a death rate of about 80%.

Ammonium carbonate as a 1% solution has been reported (12) to cause the death of hookworm ova in five days. Ammonium carbonate, ammonium chloride and saponin as 2% solutions have been found ineffective (14) against *Anguillulina* (= *Tylenchus*) *dipsaci*.

Cupric nitrate with potassium cyanide at the rate of  $\frac{1}{4}$  lb. of each in 10 gal. of water for 10 sq. ft. reduced the infection of *H. marioni* (14) and *Ditylenchus dipsaci* (16). Cupric nitrate as a 2% solution, but not as a weaker solution, was also lethal to *Tylenchus dipsaci* (15). Saponin as a 2% solution was not toxic to *T. dipsaci* (15).

Table I shows the values of the controls, which are indicated in Figs. 1 to 9 by the Roman numerals.

### *Chloropicrin*

Chloropicrin, a tear gas, which is also known as trichloronitromethane and nitrochloroform, is the most lethal chemical that has yet been tested against Sclerostome eggs or larvae. It was added to fresh faeces in quantities of 0.001 to 25.0 cc. It has a specific gravity of 1.69.

Fig. 1 shows the effects on the numbers and condition of the third-stage Sclerostome larvae finally obtained after treating fresh faeces with chloropicrin. Although 0.005 cc. very considerably reduced the number of the larvae, several reached the infective stage and many survived, even when several cubic centimetres of chloropicrin were added to the culture. This irregularity makes it very difficult to know exactly what quantity of chloropicrin is necessary to sterilize fresh horse faeces against Sclerostomes, but it is probably about 0.01 cc. to 40 gm. of faeces, equivalent to 0.043% by weight of the faeces.

These irregular results are somewhat similar to the results obtained with other chemicals that produce a lethal gas, some of which will be discussed in the next paper of this series.

The unpleasant effects on man, and even possible danger, will limit the value of this chemical for controlling Sclerostomes.

### *Aniline*

Fig. 2 illustrates the results obtained with aniline, which is also known as aminobenzene or phenylamine. Aniline was tested undiluted and in dilutions up to 1 : 1,000. It has a specific gravity of 1.022.

Undiluted, it was tested in quantities of 0.001 to 25.0 cc. Quantities of 0.005, 0.01, and 0.015 cc. each sterilized one culture, and 0.0075 cc. sterilized two cultures, but others treated with up to 0.033 cc. contained

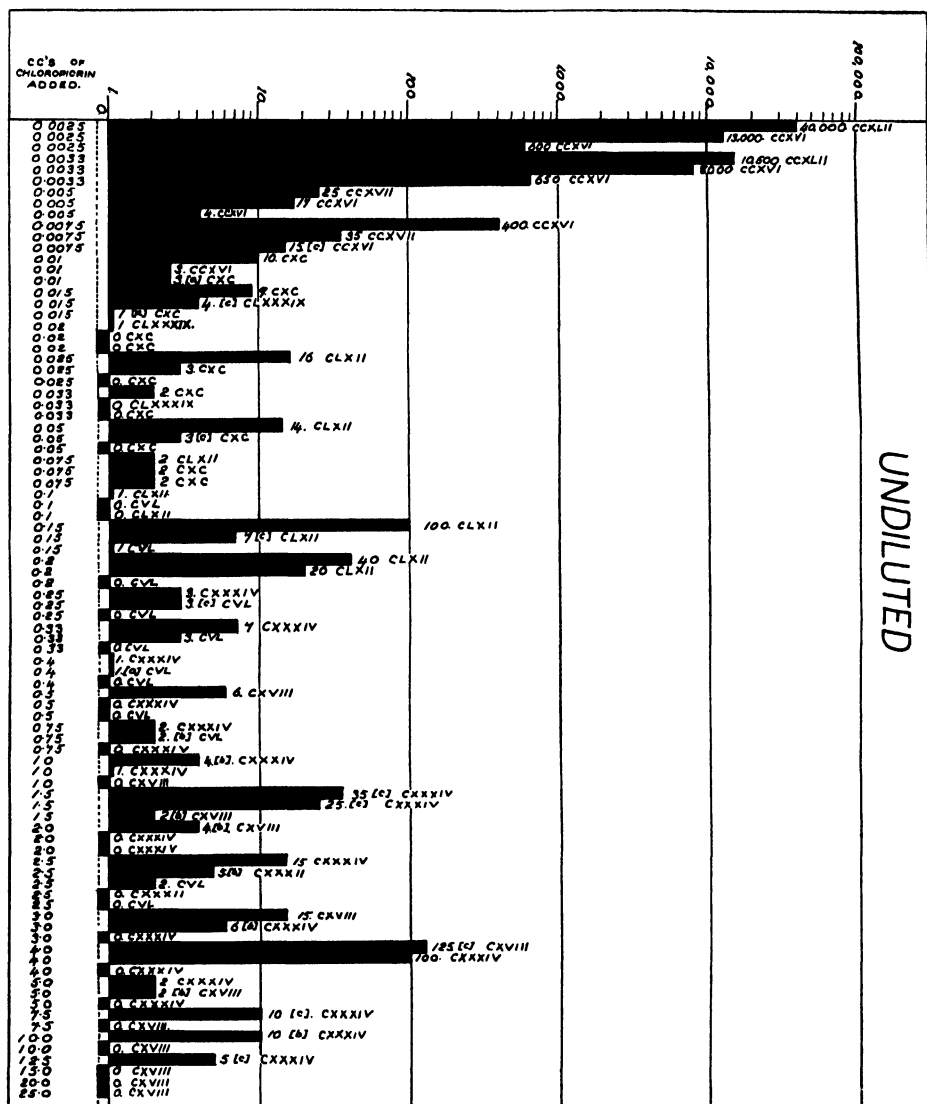


FIG. 1. Results of tests with chloropicrin, undiluted, on cultures of fresh horse faeces. Roman numerals refer to the controls shown in Table I. a, after a number means that all, or practically all the larvae were dead; b, that a considerable proportion of the larvae were dead; c, that a few larvae were dead; d, that some Sclerostome larval debris, such as sheaths, was isolated, but not counted; e, that some live larvae were isolated, some of which were probably exsheathed Sclerostomes, and, therefore, not counted. The letter e is used only when inclusion of exsheathed larvae would have significantly increased the count of ensheathed larvae. The letters have the same significance in subsequent figures.

numerous larvae, although very considerably less than in the controls. A few larvae survived in three cultures treated with 0.05 cc. With 0.075 cc. (equivalent to 0.19% of the weight of the fresh faeces) and over, the cultures were almost completely sterilized, although in some a few third-stage larvae were recovered.

TABLE I  
CONTROLS FOR CULTURES TABULATED IN FIGS. 1 TO 9

Series No.	Date cultures made	Days kept in C.T. room	Average number of larvae isolated	Series No.	Date cultures made	Days kept in C.T. room	Average number of larvae isolated
	1935				1937— <i>Conc.</i>		
XII	23 April	30	42,000	CXCI	8	48	28,000
XIV	21 May	23	29,000	CXCII	8	55	12,000
XV	21	36	18,000	CXCIV	12	51	31,000
XIX	31	32	13,000	CCVII	17 February	40	52,000
XX	31	33	12,000	CCVIII	18	49	55,000
XXII	5 June	39	25,000	CCX	23	44	35,000
XXVI	9 July	18	48,000	CCXVI	10 March	40	62,000
XXXIII	19	14	24,000	CCXVII	10	29	52,000
XXXV	22	84	23,000	CCXX	16	55	75,000
XXXVI	23	92	23,000	CCXXXVII	12 April	24	122,000
LII	22 November	25	62,000	CCXXXVIII	13	23	62,000
				CCXXXIX	15	25	62,000
	1936			CCXL	11 May	20	22,000
LXXI	16 January	22	60,000	CCXLI	18	27	35,000
LXXXII	18 February	25	16,500	CCXLII	18	27	91,000
LXXXVIII	5 March	22	15,000	CCLX	14 July	62	39,000
XCI	11	19	22,500	CCLXI	15	71	54,000
CII	1 April	20	27,500	CCLXIV	16	70	16,500
CIV	7	23	21,500	CCLXXVIII	12 October	23	28,000
CXVIII	12 May	21	2,000	CCLXXIX	13	22	44,000
CXXII	22	17	4,400	CCLXXX	14	21	37,000
CXXVII	9 June	16	11,000	CCLXXXI	15	31	44,000
CXXXII	12	22	8,500	CCLXXXII	18	28	45,000
CXXXIV	17	37	40,000	CCLXXXIII	19	27	34,000
CXXXVI	2 July	12	2,200	CCC	22 November	36	55,000
CXXXVII	2	15	5,400	CCCI	22	36	35,000
CVL	3 August	81	22,500	CCCI	23	35	24,500
CLIII	21 October	33	27,500	CCCVI	2 December	36	63,000
CLIV	22	32	36,000		1938		
CLV	22	42	76,000	CCCXX	11 January	37	31,000
CLXII	5 November	49	39,000	CCCXXI	12	36	31,000
CLXXVIII	8 December	55	100,000	CCCXXII	13	35	51,000
CLXXX	9	57	69,000	CCCXXIII	19	40	28,500
CLXXXIV	17	56	52,000	CCCXI	20	39	23,500
	1937			CCCXXXIII	18 March	61	24,000
CLXXXIX	6 January	50	28,000	CCCXXXIV	23	75	19,500
CXC	6	47	10,500	CCCLII	9 June	21	7,800 c.
				CCCLIX	12 July	20	37,000

A 1:50 aqueous solution was tested in quantities of 0.5 to 25.0 cc. From most of the cultures that were treated with up to and including 10.0 cc., a few third-stage larvae were recovered, but when 2.5 cc. or more was applied, the numbers of larvae were small (2.5 cc. of solution contains 0.049 cc. of aniline, or just over 0.12%). With the weaker solutions very similar results were obtained.

As a 1:100 solution, 4.0 cc. very considerably reduced the numbers of larvae recovered, but even with two or three times the quantity of fluid a few larvae survived (5.0 cc. is equivalent to slightly over 0.12%).

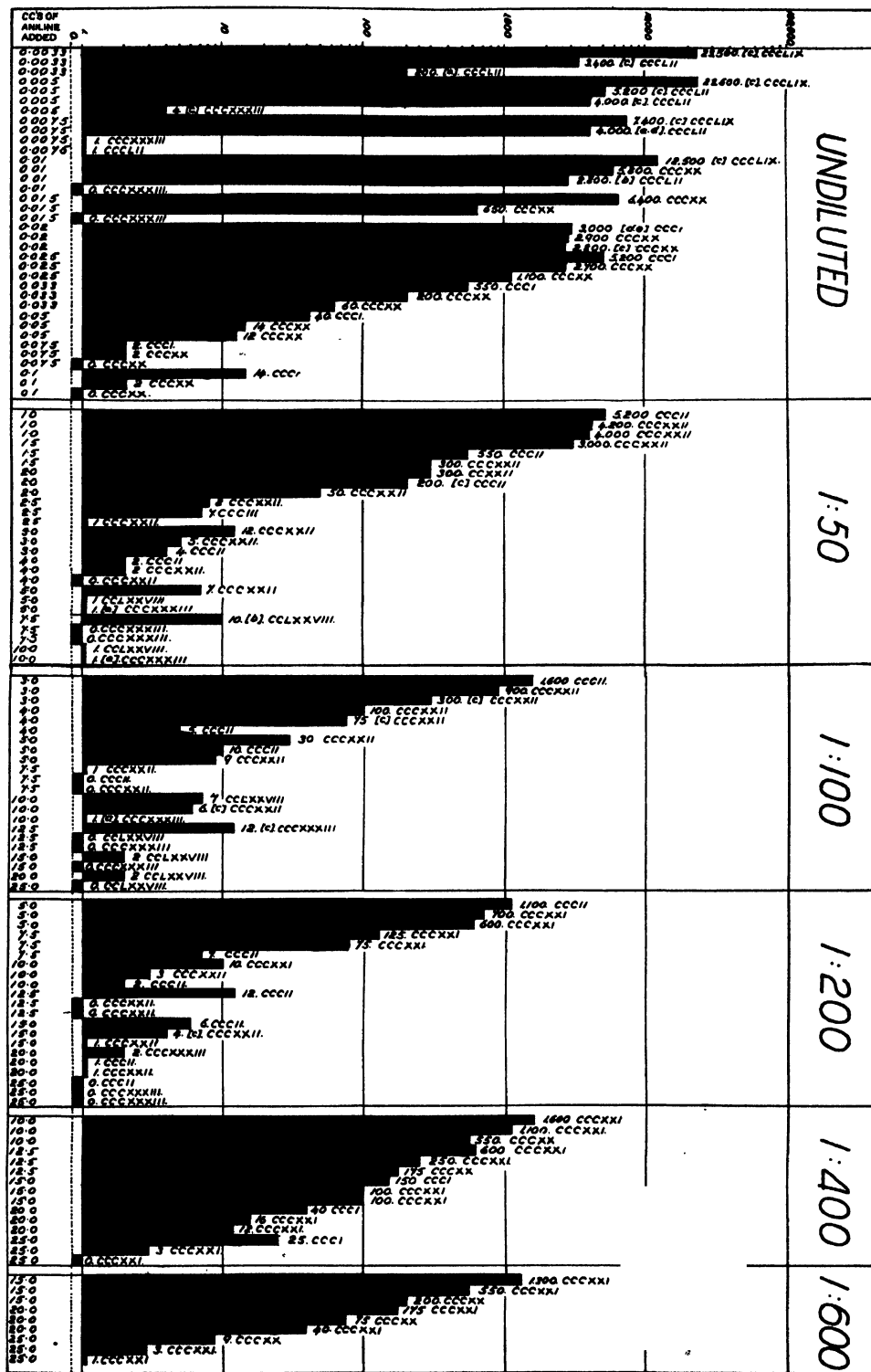


FIG. 2. Results of tests with aniline, undiluted and in solution.



and by 0.03 gm., and the numbers of larvae were very considerably reduced in all the cultures treated with 0.04 and 0.05 gm., but a very few larvae survived in some of the cultures treated with up to 1.0 gm. When larger quantities were used some reached the infective stage but subsequently died. On an average the results suggest that 0.075 gm. (equivalent to about 0.19%) is effective.

The danger to man and other mammals of the hydrocyanic gas given off by calcium cyanide will very considerably limit its use in practice.

### *Pyridine*

Fig. 4 shows the results obtained with pyridine. For this series of cultures a pure form of pyridine was used, but if it were used in practice a cheaper grade would, of course, be essential.

Pyridine, undiluted, was tested in quantities of 0.02 to 25.0 cc.; it has a specific gravity of 0.99. The addition of 0.075 cc. very considerably reduced the number of larvae and 0.1 cc. (equivalent to 0.25%), practically sterilized them, although an occasional larva survived in cultures treated with up to 0.75 cc. Larvae did not survive in the cultures treated with 1.0 cc. and over.

When diluted, there was a considerable difference in the amount of pyridine required to kill the majority compared with that necessary to kill all the larvae. When the cultures were treated with a 1 : 2 aqueous solution, 0.25 cc. reduced the larvae very considerably and 0.33 cc. (equivalent to just over 0.27%) almost caused sterilization, but 1.0 cc. and over was required to make the cultures consistently free of larvae.

Applied as a 1 : 4 solution, 0.4 cc. considerably reduced the numbers of larvae; 0.5 cc. and over almost caused sterilization, while 2.5 cc. and over caused complete sterilization with the exception of one culture treated with 4.0 cc., from which 50 larvae, many dead, were recovered (0.5 cc. is equivalent to 0.25%).

As a 1 : 8 solution, 0.75 cc. was the smallest quantity of fluid that reduced the numbers of larvae very considerably; 1.0 cc. (equivalent to 0.28%) almost caused sterilization, but 75 larvae survived in one culture treated with 2.0 cc. From all except three of the cultures treated with 4.0 cc. and over, no larvae were recovered; from each of the three exceptions one larva was obtained.

Applied as a 1 : 20 solution, 1.5 cc. caused a very marked reduction, compared with both 1.0 cc. and the controls. The addition of 2.0 to 3.0 cc. almost sterilized the cultures, while larger quantities caused complete sterilization, except in two cultures from which three and one larvae respectively were recovered (2.0 cc. is equivalent to 0.24%).

The results obtained with a 1 : 50 solution are not illustrated. The addition of 4.0 cc. effected a marked reduction in the numbers of larvae, 5.0 cc. (equivalent to 0.24%) further reduced them to a negligible number, while none was recovered from any of the cultures treated with 7.5 cc. or over.



When added to the cultures as a 1 : 100 solution, 7.5 and 10.0 cc. reduced the numbers, the latter considerably, while 12.5 cc. and over practically sterilized the cultures (12.5 cc. contains about 0.33%).

Pyridine was also tested as a 1 : 200, a 1 : 300, a 1 : 400 and a 1 : 500 aqueous solution. The results are not illustrated.

Applied as a 1 : 200 solution, 15.0 and 20.0 cc. reduced the numbers considerably, while 25.0 cc. (equivalent to 0.31%) further reduced them to 50, 5, and 2. The weaker solutions were proportionately less effective.

An average of these results shows that about 0.27% of pyridine will sterilize fresh faeces under conditions similar to those existing for these cultures.

#### *Ammonium Carbonate*

The results obtained with ammonium carbonate are illustrated in Fig. 5.

With this chemical also, a few larvae escaped in cultures which had been treated with quantities considerably larger than that which caused death to most of the eggs and larvae.

Ammonium carbonate was applied dry in quantities of 0.2 to 8.0 gm. One culture was sterilized by 0.5, two by 0.75, and all by 1.0 gm., but in the cultures to which 1.5 gm. was added a few larvae survived. These results suggest that about 1 gm. (or 2.5%) of dry ammonium carbonate is necessary to cause sterilization.

Applied as a 1 : 2 solution, 2.0 cc. (equivalent to 1.9%) was effective. Again a few larvae reached the infective stage when larger quantities were added but most of these subsequently died.

When added to the cultures as a 1 : 4 solution, 4.0 cc. containing 0.89 gm. (equivalent to 2.2%) was effective; 2.5 and 3.0 cc. in one culture of each almost caused sterilization.

The results obtained with a 1 : 8 solution were somewhat irregular. Two cultures were sterilized by 5.0 cc., but 100 active larvae were isolated from the third. From the three cultures treated with 7.5 cc., respectively 500 dead, 200 active and 40, many of them dead, larvae were isolated. From one of the cultures treated with 12.5 cc., 175 dead larvae were obtained. An average of these results suggests that about 7.5 cc. (equivalent to 2.25%) will cause sterilization.

Applied as a 1 : 20 solution, the cultures treated with 15.0 cc. (equivalent to slightly over 1.8%) were almost sterilized, while 25.0 cc. was completely effective.

Added as a 1 : 50 solution, larger quantities considerably reduced the numbers of the larvae but did not cause sterilization.

The results suggest that on an average, ammonium carbonate must be used at the rate of about 2.1% of the weight of fresh faeces to produce sterilization against the free-living stages of Sclerostomes.

#### *Ammonium Chloride*

Fig. 6 illustrates the results obtained with ammonium chloride, which is also known as white salammoniac. It was tested dry in quantities of 0.2



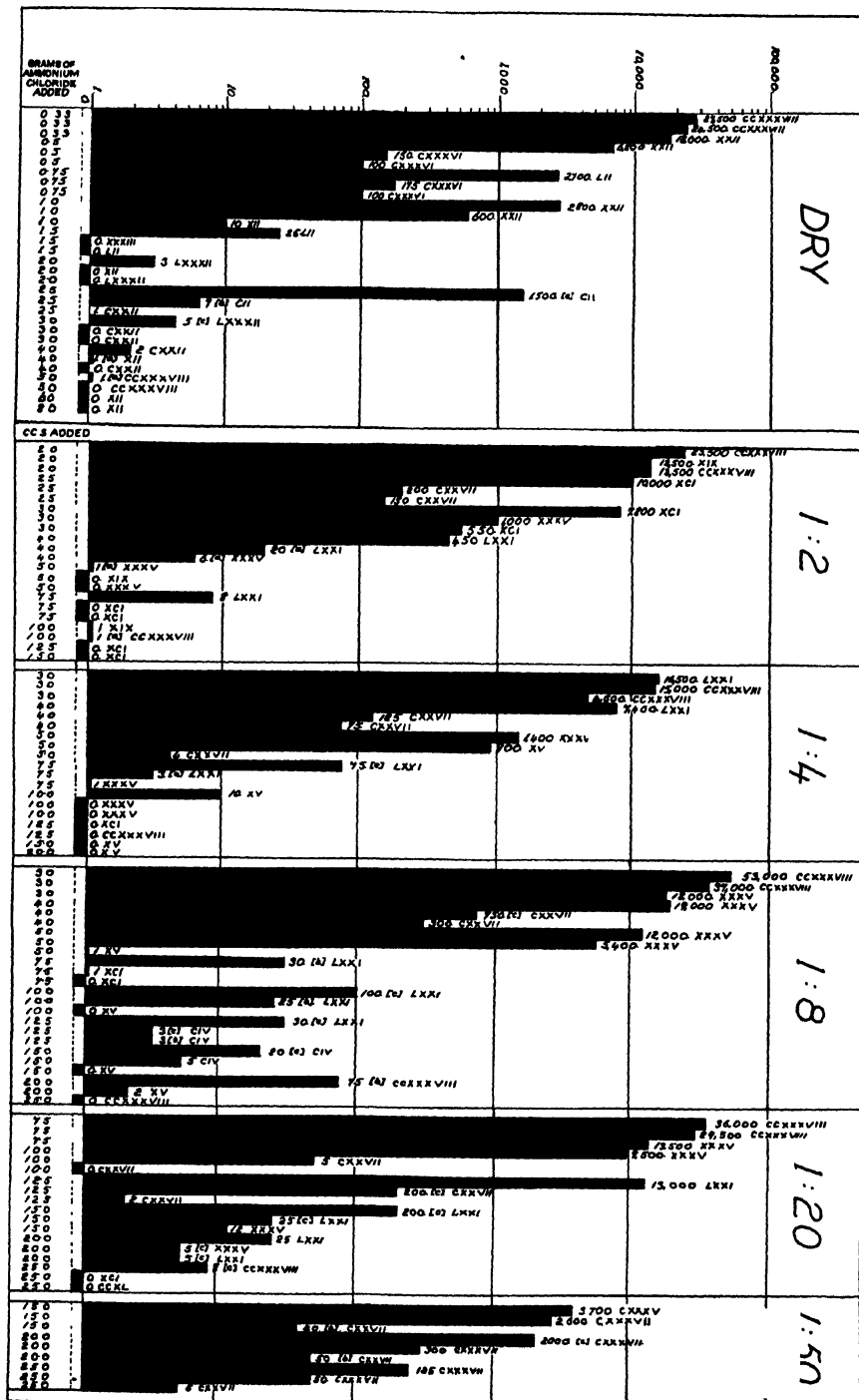


FIG. 6. Results of tests with ammonium chloride, dry and in solution.

to 8.0 gm. The results were irregular but it is probable that 1.5 gm. (equivalent to 3.75%) is approximately the amount necessary to sterilize fresh faeces. As one culture was almost sterilized by 1.0 gm. and all the cultures were almost sterilized by 1.5 and 2.0 gm., the culture that had been treated by 2.5 gm. and from which 1,500 larvae, a few dead, were recovered, need not be considered in making this estimate.

When added as a 1 : 2 solution, 5.0 cc. and over was effective and 4.0 cc. sterilized two cultures and reduced the number of larvae in the other (5.0 cc. contains about 4.7%).

When the cultures were treated with a 1 : 4 solution, 4.0 cc. considerably reduced the number of larvae in two cultures, and 5.0 cc. almost sterilized one culture. Two out of three cultures were sterilized by 7.5 cc., but 75 larvae, including a few dead, were isolated from the third. Averaging these results suggests that 7.5 cc., containing about 1.6 gm. (equivalent to 4.0%) is the amount of ammonium chloride, applied as a 1 : 4 solution, necessary to cause sterilization.

Although the results obtained with a 1 : 8 solution were more irregular, they suggest it is proportionately more effective than the stronger solutions. The addition of 4.0 cc. considerably reduced the numbers of larvae in two cultures, and 5.0 cc. sterilized one. In the series of cultures to which from 7.5 cc. (equivalent to 2.25%) to 20.0 cc. were added, some were sterilized, and from others a few larvae (mostly dead) were isolated.

Applied as a 1 : 20 solution, one culture was completely and one was nearly sterilized by 10.0 cc. Adding 12.5 cc. had a similar effect. From the cultures treated with 15.0 cc. and over, a few larvae were isolated, of which a few were dead (15.0 cc. is equivalent to slightly over 1.8%).

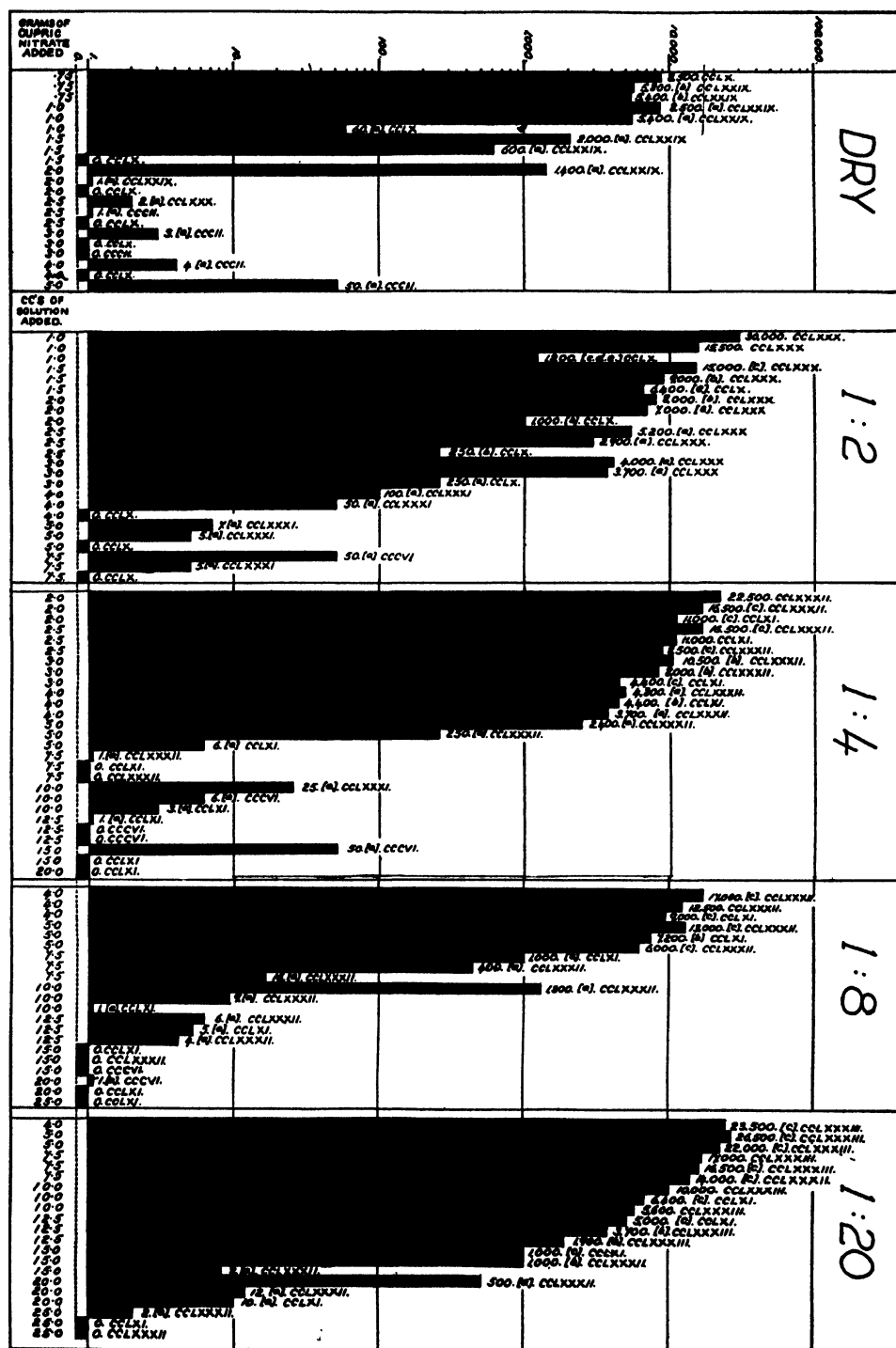
Added as a 1 : 50 solution, the larger quantities greatly reduced the numbers of larvae, but did not completely sterilize any cultures.

An average of these small-scale tests suggests that about 3.3% of ammonium chloride will sterilize fresh faeces against *Sclerostomes*, but that less is effective if it is applied as a medium strength solution.

### *Cupric Nitrate*

Fig. 7 illustrates the effects of cupric nitrate ( $\text{Cu}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ ) on the numbers and condition of the third stage ensheathed *Sclerostome* larvae from faeces that have been treated when fresh. Cupric nitrate, like many metallic sulphates and chlorides, may allow the larvae to reach the infective stage but subsequently may cause their death. This makes cupric nitrate difficult to evaluate, because it is impossible to know whether these larvae might have been capable of developing further if they had been swallowed by a suitable host, soon after they reached the third or infective stage.

Cupric nitrate was tested dry in quantities of 0.25 to 8.0 gm. One-half gram and less had little, if any, apparent effect on the vitality of the third-stage larvae, but 0.75 gm. caused many to die, and 1.0 gm. and over killed



them all. On an average, approximately 2.0 gm. (or 5.0%) of cupric nitrate was necessary to free from larvae the 40 gm. of fresh faeces.

When applied as a 1 : 2 solution, some of the larvae which reached the infective stage were killed by 1.5 cc., many were killed by 2.0 cc. and practically all by 2.5 cc. About double the latter amount made the cultures practically free of larvae, and 4.0 cc. greatly reduced the numbers (5.0 cc. is equivalent to about 5.25%).

Less than 2.0 cc. of a 1 : 4 solution appeared to have no effect on the vitality of the larvae which reached the infective stage; many died when 3.0 cc. was added and 4.0 cc. was lethal to the great majority. The cultures were practically free of larvae when 7.5 cc. (equivalent to 4.5%) was added.

When 4.0 and 5.0 cc. of a 1 : 8 solution were added, a few of the larvae which reached the third stage, subsequently died; when 7.5 cc. was added they all died. If the numbers of larvae isolated from the cultures treated with 7.5, 10.0 and 12.5 cc. are averaged, they suggest that 10.0 cc. (equivalent to 3.0%) is effective in making the cultures almost free from larvae when applied as a 1 : 8 solution.

Applied as a 1 : 20 solution, 20.0 cc. (equivalent to almost 2.5%) is probably effective in sterilizing the faeces. When 12.5 and 15.0 cc. were added, many larvae that reached the infective stage died, but when less was added only a few died.

The addition of the larger quantities of a 1 : 50 solution of cupric nitrate reduced the numbers of larvae, but did not sterilize the cultures.

An average of the results obtained with cupric nitrate suggests that to make the faeces almost free of third-stage larvae approximately 4.0% must be added to the fresh faeces, but that less is effective if it is applied as a medium strength solution, and that a half, or two-thirds of this quantity will cause the death of the larvae after they have developed to the infective stage, although treated in the egg stage.

### *Ammonium Nitrate*

The results obtained with ammonium nitrate are illustrated in Fig. 8. This chemical was applied dry in quantities of 0.25 to 8.0 gm. If the effectively sterilized cultures made with 1.5, 2.0 and 3.0 gm. of ammonium nitrate are averaged with those which were made with the same quantities, but were not effectively sterilized, the results suggest that between 2.0 and 2.5 gm. (equivalent to about 6%) is the amount necessary to effect sterilization.

Applied as a 1 : 2 solution, 4.0 cc. sterilized one culture, 5.0 cc. sterilized two, and 7.5 cc. and over sterilized practically all the cultures (7.5 cc. is equivalent to about 7.0%). As some cultures were sterilized by 4.0 cc. and 5.0 cc. this estimate would probably be found to be too high.

Applied as a 1 : 4 solution, 7.5 cc. (containing about 4.1%) effectively sterilized the cultures, 5.0 cc. did not.

Added to the cultures as a 1 : 8 solution, the results are again irregular, but they suggest that 15.0 cc. (equivalent to 4.5%) is definitely, and 12.5 cc. may be, effective.

Applied as a 1 : 20 solution, 20.0 cc. very considerably reduced the numbers of the larvae in two cultures and sterilized one culture. The addition of

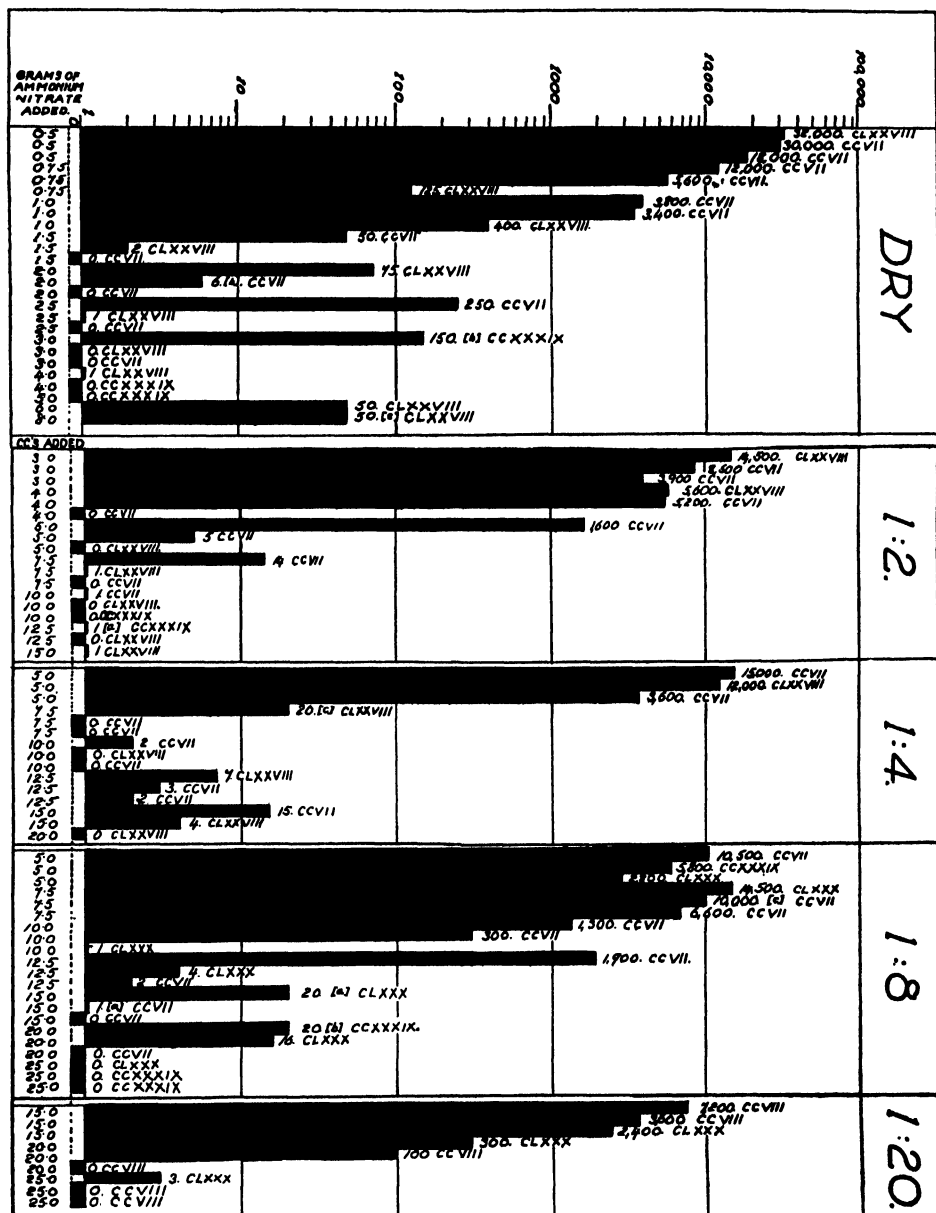


FIG. 8. Results of tests with ammonium nitrate, dry and in solution.

25.0 cc. (equivalent to slightly over 3.0%) of the same solution sterilized three cultures.

If the results are averaged, they suggest that approximately 4.75% of the ammonium nitrate is required to sterilize fresh faeces. They also show that ammonium nitrate should be used as a medium strength solution, when two-thirds of this amount may be effective.

### Ammonium Sulphide

Ammonium sulphide was tested as "yellow" solution, containing 15% of ammonium sulphide in 85% of water. The results obtained with it are illustrated in Fig. 9. Without further dilution, 2.0 and 2.5 cc. each practically sterilized one culture, 3.0 cc. sterilized two cultures but 700 larvae were isolated from a third, while 4.0 cc. and over was effective.

When diluted with twice its volume of water, 7.5 cc. and over sterilized the cultures.

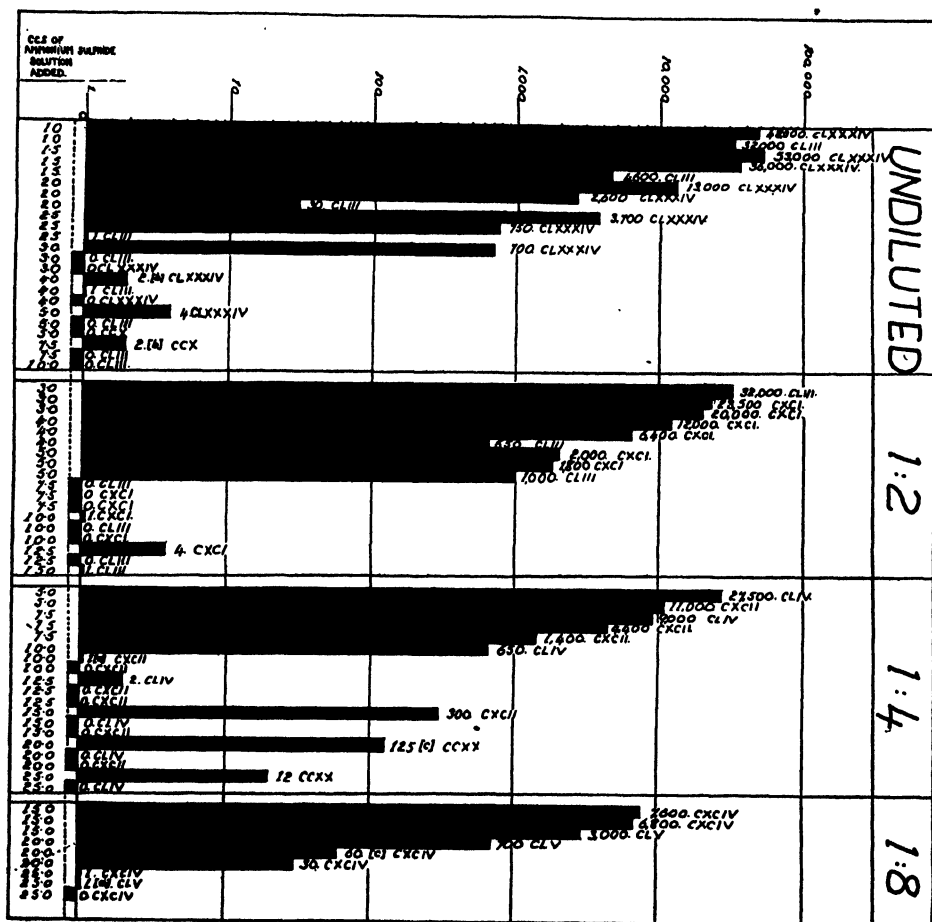


FIG. 9. Results of tests with ammonium sulphide, as a 15% solution and diluted further.

When four times its volume of water was added, two cultures were sterilized by 10.0, by 15.0 and by 20.0 cc., but three were sterilized by 12.5 cc.

When the dilution was increased to eight times the volume of water, 25.0 cc. caused sterilization, and 20.0 cc., reduction in the number of larvae.

Sterilization did not occur when the dilution with water was increased to 20 times.

The average of these results suggests that about 3.0 cc. of the yellow solution (containing 15% of ammonium sulphide) is effective in sterilizing 40.0 gm. of fresh faeces. It is probably slightly more effective when the solution is further diluted with up to eight times its volume of water.

### Saponin

Saponin ( $C_{27}H_{47}O_{16}N$ ) was tested dry and as 1 : 2, as 1 : 4, as 1 : 8, and as 1 : 20 aqueous "solutions". In some of the cultures made with saponin mixed in the faeces, both dry and in solution, the number of the larvae that were isolated was reduced, in a few cultures they were killed, and in a few they had exsheathed. As there was apparently little correlation between the quantity of saponin and the reduction in numbers or alterations of condition of the larvae, it is probable that saponin has little, if any, lethal action chemically, although its physical properties may affect the larvae. The possibility that saponin might add to the value of any lethal chemical that is difficult to bring in contact with the larvae, has not yet been tested.

The results with saponin were so irregular that they have not been illustrated.

### Conclusions

Chloropicrin is the most lethal chemical that has yet been tested against Sclerostomes in fresh faeces, but its use, like that of calcium cyanide, which also is extremely lethal, will be limited by the difficulty of confining the gas and by its effects on mammals. If a sufficiently cheap grade of aniline or pyridine were available, their use might be practicable on many farms, especially if they could be used for fly control at the same time. The practicability of using ammonium carbonate, ammonium chloride, cupric nitrate, and ammonium sulphide to treat the top and sides of a midden, would depend largely on their cost, compared with that of comparable nitrogenous fertilizers. The price factor also applies to the practicability of using ammonium nitrate, which is slightly more lethal than potassium nitrate and sodium nitrate. Saponin probably has no chemical value as a lethal agent.

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## LAND AND FRESHWATER MOLLUSCA FROM WESTERN ONTARIO<sup>1</sup>

BY FRANK COLLINS BAKER<sup>2</sup>

In the summer of 1931, Dr. A. R. Cahn, then assistant professor of Zoology in the University of Illinois, made a canoe trip over the lakes and rivers of the International Boundary between Canada and the United States and the waters immediately to the north and east of this boundary. In July, 1935, Dr. Cahn made a similar trip through a portion of the Lake of the Woods region. Dr. R. G. Lindenberg, a graduate student in the Department of Zoology, made collections in the Quetico Provincial Park, Rainy River District. The latter collection consisted principally of land molluscs while that of Dr. Cahn was made up largely of freshwater species. The present paper may be considered supplementary to that of Baker and Cahn (6) on freshwater Mollusca from central Ontario. Some of the data on Planorbidae were published by Baker (4).

In the paper by Baker and Cahn, the material listed was collected in Thunder Bay district and Rainy River district. In the present paper the itinerary was largely confined to the Rainy River district and included the following lakes: Crooked Pine, Pickerel, Sturgeon, Basswood, Agnes, Louisa, Knife, Kahnipiminanikok, Saganagons, and Saganaga. Many smaller lakes and connecting waters were also visited.

The 1935 trip made by Dr. Cahn was in the Lake of the Woods region, Kenora district. In outline the itinerary was as follows: Lakes Kakagi, Cedartreë, Flint, Cameron, Otterskin, Hillside, Horseshoe, Pipestone, Kishkutena, Sabaskong Bay, Miles Bay, Tug Channel, and Whitefish Bay. A portion of the trip was around the Aulneau Peninsula, on the east side of Lake of the Woods.

The vast area of Lake of the Woods, with the myriads of small islands in the lake and the hundreds of lakes in the immediate region, is comparatively little known conchologically. Dawson's paper (7) is the most extensive report on the fauna of this region and lists 24 species, 14 gastropods and 10 pelecypods. The Cahn collection contained 16 gastropods and 5 pelecypods. Many small clams of the Sphaeriidae were not contained in the Cahn collection and some small gastropods listed by Dawson were also missing. Lake of the Woods is notable as being one of the type localities of Say's *Planorbis corpu-*

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*lentus*, one of the finest species of the genus *Helisoma*. The variety *multicostatum* also occurs in the lake (4).

A noteworthy contribution from the Cahn collection is the presence of *Helisoma campanulatum collinsi*, a new race of *campanulatum* abundant in the Lake of the Woods. A careful study of the Lake of the Woods region by a trained conchologist with abundance of time would doubtless increase the molluscan fauna to more than 50 species and races.

The present paper lists 22 species and races not included by Baker and Cahn (6).

The writer is greatly indebted to both Dr. Cahn and Dr. Lindenberg for the collections made in Ontario, which have been given to the University of Illinois and form a part of the collection of Mollusca of the Museum of Natural History. Thanks are due to Dr. W. J. Clench, of the Museum of Comparative Zoology, for the identifications of certain *Physa*, and to Dr. Harold A. Rehder, of the U.S. National Museum, for the identification of certain *Succinea*.

## Pelecypoda

### FAMILY UNIONIDAE

#### *Anodonta grandis footiana* Lea

Rainy River district: Baird Lake; Keats Lake; Athelstane Lake; Crooked Pine Lake; Basswood Lake; Kahnipiminanikok Lake; between Saganagons and Othermans Lake; rapid water between Saganagons and Kahnipiminanikok Lakes; Greenwood Lake; Camel Lake; Ogish-ke-muncie Lake; Mowe Lake; Silver Lake; Lasper Lake; Sark Lake; Fern Lake; Brent Lake. Thunder Bay district: Chief Peter Lake. Kenora district (Lake of the Woods): Rabbit Point, west of Sabaskong Bay; McPherson Island, near Sabaskong Bay; Astron Bay, north side Aulneau Peninsula; Turtle Portage, north of Sabaskong Bay; Horseshoe Lake, east of Kakagi Lake; southeast side Sabaskong Bay, on small island; Sammy Lake, east of Kakagi Lake.

This *Anodonta* is the most abundant form of the genus in western Ontario. It is more elongated and less high than the specimens from the type locality in Winnebago Lake, Wisconsin, but is well within the range of variation of the race (1, p. 157). An average adult specimen measures well over 100 mm. in length and 50 mm. in height. The colour is dark yellowish-brown, sometimes with a decided greenish tinge. The later Cahn records widely extend the distribution of the race in Ontario.

#### *Anodonta grandis plana* Lea

Thunder Bay district: Waikwabauona River, flowing into Northern Light Lake. Rainy River district: river between Crooked Pine and Chief Peter Lakes; Pickerel River.

A large *Anodonta*, common to abundant in the first two localities cited above, appears referable to the creek form of *grandis*. It is large, attaining a length of over 100 mm., the shell is elongated with pointed posterior end. This race was not observed in the material listed by Baker and Cahn (6, p. 44).

*Anodonta kennicottii* Lea

Rainy River district: Basswood River rapids; McKenzie Lake.

*A. kennicottii* does not appear to be as abundant in Ontario as it is in Minnesota and Wisconsin.

*Anodonta marginata* Say

Rainy River district: Kekequebic Lake; Ogish-ke-muncie Lake; Crooked Pine Lake; Greenwood Lake. Thunder Bay district: Northern Light Lake. Kenora district (Lake of the Woods): Turtle Portage, north of Sabaskong Lake.

*A. marginata* appears more widely distributed in Ontario than was indicated by the collections previously made. Its greenish, fragile shell can scarcely be mistaken for any other species.

*Anodontoides ferussacianus* (Lea)

Rainy River district: Kashaboiwe Lake. Kenora district (Lake of the Woods): McPherson Island, near Sabaskong Bay.

*A. ferussacianus* was not observed in the material previously collected by Dr. Cahn in Ontario. Only two specimens were found in the material now under observation, and the species is probably to be considered as rare in this part of Ontario.

*Lasmigona compressa* (Lea)

Rainy River district: Kashahpiwigamak Lake; Kabwawiagamak River; river between Crooked Pine Lake and Chief Peter Lake.

*L. compressa* was not contained in the collections previously made by Dr. Cahn. The three localities listed above indicate that it occurs in western Ontario although, apparently, not abundantly, since in two of the localities only broken and partly worn shells were obtained. The specimens from the river between Crooked and Chief Peter Lakes were living and quite characteristic of the species. The largest specimen measures 87 mm. in length. The colour is dark olive with indistinct rays. Simpson (Cat. p. 482) gives the range as north to Wisconsin, but the Canadian records indicate a much more northern range than this.

*Lampsilis superiorenensis* (Marsh)

Thunder Bay district: Obadinaw River. Rainy River district: Kabwawiagamak River; Kahnipiminanikok Lake; McKenzie Lake; Kashahpiwigamak Lake; Crooked Pine Lake; river between Crooked Pine and Chief Peter Lakes. Kenora district (Lake of the Woods): southeast side Sabaskong Lake, on small island; Rabbit Point, west of Sabaskong Bay.

Although bearing a strong resemblance to some forms of *L. siliquoidea* (Barnes), *L. superiorenensis* appears to be sufficiently distinct and appears to be the prevailing *Lampsilis* in this part of Ontario, as remarked in a previous paper.

*Lampsilis ventricosa canadensis* (Lea)

Rainy River district: Fern Lake.

One female specimen of this race is contained in the Cahn collection. It is 78 mm. in length and 49 mm. in height and is greenish yellow in colour. This race should be found more abundantly than the collections would indicate.

## FAMILY SPHAERIIDAE

*Sphaerium sulcatum* (Lamarck)

Rainy River district: Kashahpiwigamak River; between Saganagons Lake and Othermans Lake; Camel Lake; Mack Lake; lake west of West Lake and north of South Lake.

*S. sulcatum* was not abundant in the collections from Ontario previously listed (6, p. 46). It was common in several of the localities listed above.

*Sphaerium fallax* Sterki

Rainy River district: Greenwood Lake; Crooked Pine Lake; Kashaboiwe Lake. Thunder Bay district: South Arm, Saganaga Lake; Waikwabauona River, near Northern Light Lake. Kenora district (Lake of the Woods): Rabbit Point, west of Sabaskong Bay.

*S. fallax* appears to be an abundant species of this genus in Ontario. It is variable but apparently quite distinct from its nearest relative, *sulcatum*.

*Sphaerium striatinum* Lamarck

Thunder Bay district: Northern Light Lake and Waikwabauona River, near Northern Light Lake.

A *Sphaerium* which appears to be this species is very abundant in the localities listed.

*Sphaerium rhomboideum* (Say)

Rainy River district: between Saganagons and Othermans Lakes, one typical specimen.

Apparently this *Sphaerium* is rare in Ontario.

*Musculium securis* (Prime)

Thunder Bay district: Waikwabauona River.

Abundantly represented in the collection.

*Pisidium*, species indet.

Rainy River district: Otter Track Lake.

A very small species of this perplexing genus. Since the death of Dr. Victor Sterki no one has taken up the study of these small bivalves, and the writer does not feel competent to attempt the identification of this species.

## Gastropoda

### FAMILY VALVATIDAE

#### *Valvata tricarinata* (Say)

Kenora district (Lake of the Woods): Kennedy Island. Rainy River district: Sturgeon Lake.

The typically tricarinate form of the species.

#### *Valvata sincera nylanderi* Dall

Kenora district (Lake of the Woods): Kennedy Island.

Several characteristic specimens occurred in this collection. This is apparently the first record of *V. nylanderi* from this part of Ontario.

#### *Valvata lewisi ontariensis* F. C. Baker

The specimens listed as a discoidal form of *V. lewisi helicoidea* (6, p. 49) have been found to constitute a distinct race of *lewisi*. The types of this race are from Shakespeare Lake, near Nipigon Lake, Ontario, where the race occurs in some abundance (Nautilus, 44 : 119. 1931). The Cahn specimens came from Kimmewin Lake, near Drayton.

### FAMILY VIVIPARIDAE

#### *Campeloma*, species indet.

Rainy River district: Basswood River. Thunder Bay district: Northern Light Lake, east of Saganaga Lake. Kenora district (Lake of the Woods): McPherson Island.

Baker and Cahn (6) identified a *Campeloma* from Hamilton Lake as *milesii* (Lea). Until the whole genus has been carefully examined anatomically it is unsafe to identify positively these northern forms. Some specimens approach *milesii* in form and others might be referred to *decisum*. This genus is probably abundantly represented in the northern lakes but careful search is required to find them.

### FAMILY AMNICOLIDAE

#### *Amnicola limosa superiorenensis* F. C. Baker

Rainy River district: Kahnipiminanikok Lake; Sturgeon Lake; Kasha-boiwe Lake; lake west of West Lake and north of South Lake. Kenora district (Lake of the Woods): Kennedy Island.

The *Amnicola* referred to *porata* by Baker and Cahn (6, p. 49) is *superiorenensis*, which is the common *Amnicola* in the lakes of western Ontario. The *Amnicola* listed as *porata*, from Prince Albert National Park, Saskatchewan, is also referable to *superiorenensis* (5, p. 115). Typical *porata* must conform to the shells from the original locality, Cayuga Lake, N.Y. See Baker (1, p. 101) for definition of *superiorenensis*.

## FAMILY LYMNAEIDAE

*Lymnaea stagnalis jugularis* Say

Rainy River district: Basswood Lake; Snodgrass Lake; Kashabowi Lake; Emerald Lake; Carp Lake; Knife Lake; Mercutio Lake; rapid water between Saganagons and Kahnipiminanikok Lakes. Thunder Bay district: Lac des Mille Lacs swamp. Kenora district (Lake of the Woods): Otterskin Lake.

This typical form of the American *L. stagnalis* is apparently common in the lakes of Ontario.

*Lymnaea stagnalis lillianae* F. C. Baker

Thunder Bay district: Lac des Mille Lacs; Lac des Mille Lacs swamp.

This race appears to be common in certain lakes of Ontario. See the paper by Baker and Cahn (6) for additional records in Ontario.

*Lymnaea stagnalis sanctaemariae* Walker

Thunder Bay district: Lac des Mille Lacs swamp; Chief Peter Lake. Rainy River district: Crooked Pine Lake; Athelstane Lake; Emerald Lake; between Saganagons and Othermans Lakes.

The specimens referred to the race *sanctaemariae* have a somewhat longer spire than specimens from St. Mary's River in Michigan, but they all have the peculiar aperture of the race. The Ontario specimens vary toward *lillianae* on one hand and *jugularis* on the other. There is usually little difficulty in separating the three races of this polymorphic species.

*Stagnicola lanceata* (Gould)

Thunder Bay district: Cat Lake; Lac des Mille Lacs. Rainy River district: Mercutio Lake; Snodgrass Lake; rapid water between Saganagons and Kahnipiminanikok Lakes; lake west of West Lake and north of South Lake; Iron Lake; Knife Lake; Little Knife Lake.

*Stagnicola lanceata* is apparently widely distributed in western Ontario. The type locality is Pic Lake, north of Lake Superior. It was not included in the collections reported in 1931 (6).

*Stagnicola cf. nasoni* (F. C. Baker)

Rainy River district: Keats Lake.

A few specimens of a *Stagnicola* apparently referable to *nasoni* were collected in 1932 by Dr. Cahn. The colour is greenish-horn upon which the rest varices show as white vertical lines. The Knife Lake specimens are not exactly like the form found on the shores of the Great Lakes, and more specimens might show it to be a recognizable race. It is tentatively referred to *nasoni* at present.

*Stagnicola emarginata kempfi* Baker and Cahn

Rainy River district: Basswood River; Kahnipiminanikok Lake; Carp Lake; McAree Lake; rapid water between Saganagons and Kahnipiminanikok Lakes. Kenora district (Lake of the Woods): McPherson Island. Minnesota: St. Louis Co., Iron Lake.

A further study of this race of *Stagnicola* has led the writer to relate it to *emarginata* rather than to *catascopium*, as indicated in the original description (6, p. 53, Plate II). It appears to be more intimately related to typical *emarginata*, but differs from that form in several particulars. This *Stagnicola* appears to be the dominant form of the genus in western Ontario. In Basswood River it is very abundant.

*Stagnicola caperata* (Say)

Rainy River district: Mack Lake.

Two specimens of this small lymnaeid were collected by Dr. Lindenberg in a swale near the lake.

*Bulinnea megasoma* (Say)

Thunder Bay district: Chief Peter Lake; Lac des Mille Lacs swamp. Rainy River district: Kashaboiwe Lake; Emerald Lake; Mowe Lake; Little Knife Lake; between Saganagons and Othermans Lakes; Athlestone Lake; Basswood Lake; Knife Lake; South Arm, Saganaga Lake; Baird Lake; Mercutio Lake; Snodgrass Lake; Keats Lake; lake west of West Lake and north of South Lake. Kenora district (Lake of the Woods): Otterskin Lake. Minnesota: St. Louis Co., Iron Lake.

*Bulinnea megasoma* is very abundant in Ontario, where it appears to reach its maximum in development. Most of the specimens are brownish with a greenish tinge and the interior of the aperture is usually rich purple. Average specimens are about 45 mm. in length.

FAMILY PLANORBIDAE

*Helisoma anceps* (Menke) 1830 (= *Planorbis antrosus* Conrad, 1834)

Rainy River district: Basswood River; lake west of West Lake and north of South Lake (very abundant); Mackenzie Arm, Lake Kahnipiminanikok; Emerald Lake. Kenora district (Lake of the Woods): McPherson Island; Horseshoe Lake.

This species of *Helisoma*, long known under the name *Planorbis bicarinatus* and later as *Helisoma antrosus*, is very abundant in some of the lakes of Ontario. The material examined shows little variation from the typical form. In Emerald Lake, specimens had the wrinkled body whorl to which Currier gave the name *corrugatus*. Typical *anceps* were not recorded by Baker and Cahn (6).

*Helisoma anceps sayi* (F. C. Baker)

Rainy River district: lake west of West Lake and north of South Lake.

About 5% of the specimens in this lake are referable to the race distinguished as *sayi*, first noted in Wisconsin in Tomahawk Lake (1, p. 322). In this race the basal carina is near the peripheral edge of the whorl. In many lakes *sayi* only is represented, but in others it is mixed with typical *anceps*.

*Helisoma anceps royalense* (Walker)

Rainy River district: Carp Lake; Mercutio Lake; Camel Lake; Basswood River rapids. Thunder Bay district: Northern Light Lake.

The race of *anceps* called *royalense* was not common in the collections made more recently by Dr. Cahn. It was more abundant in the lakes in Thunder Bay district reported upon in 1931 (6, p. 55).

*Helisoma anceps rushi* Var. Nov. (Fig. 1)

In northern Minnesota and southern Ontario a form of *anceps* occurs that was previously referred to *jordanense* Winslow. An examination of material of *jordanense* from the type lot indicates clearly that the form living in Canada and Minnesota is not this race but one hitherto unrecognized. *Jordanense* (Fig. 1) is a Pleistocene fossil, the specimens being white and chalky. They were dredged from the bottom of Lake Charlevoix, Charlevoix Co., Mich., apparently from a marl deposit. *Lymnaea bakeri* Walker is from the same deposit.

*Rushi* differs from *jordanense* in the comparative lesser height of the body whorl behind the aperture, more rounded contour of the body whorl laterally, the less distinct carina at the edge of the whorl on the base and the rounded, not sharply angled margin of the spire depression. The recent race is also smoother and quite polished, while the sculpture of *jordanense* consists of strong, prominent lines of growth. *Rushi* is probably the living descendant of *jordanense*. Types of *rushi* are from Toad Island, Georgian Bay, Ontario, and were collected in two feet of water. Types, No. Z25259, Museum of Natural History, Univ. of Ill. Holotype: height 7.5; diameter 14.0; aperture height, 6.3; diameter 4.2 mm.

The new race has been seen from Minnesota and from the following localities in Ontario, Canada:

Rainy River district: Keats Lake; Kashaboiwe Lake; Fern Lake. Kenora district (Lake of the Woods): Otterskin Lake; Kennedy Island, near Whitefish Bay.

*Helisoma trivolvis* (Say)

Rainy River district: Athelstane Lake; Snodgrass Lake; Kahnipiminanikok Lake.

In the Baker and Cahn paper (6), *trivolvis* is recorded from Thunder Bay district as not uncommon. The specimens herein recorded from Rainy River district are fine typical examples. The species is widely distributed in western Ontario.

*Helisoma trivolvis macrostomum* (Whiteaves)

Thunder Bay district: South Arm, Saganaga Lake. Rainy River district: Kashaboiwe Lake; Mercutio Lake; Snodgrass Lake; between Saganagons and Oothermans lakes; between Saganagons and Kahnipiminanikok Lakes.

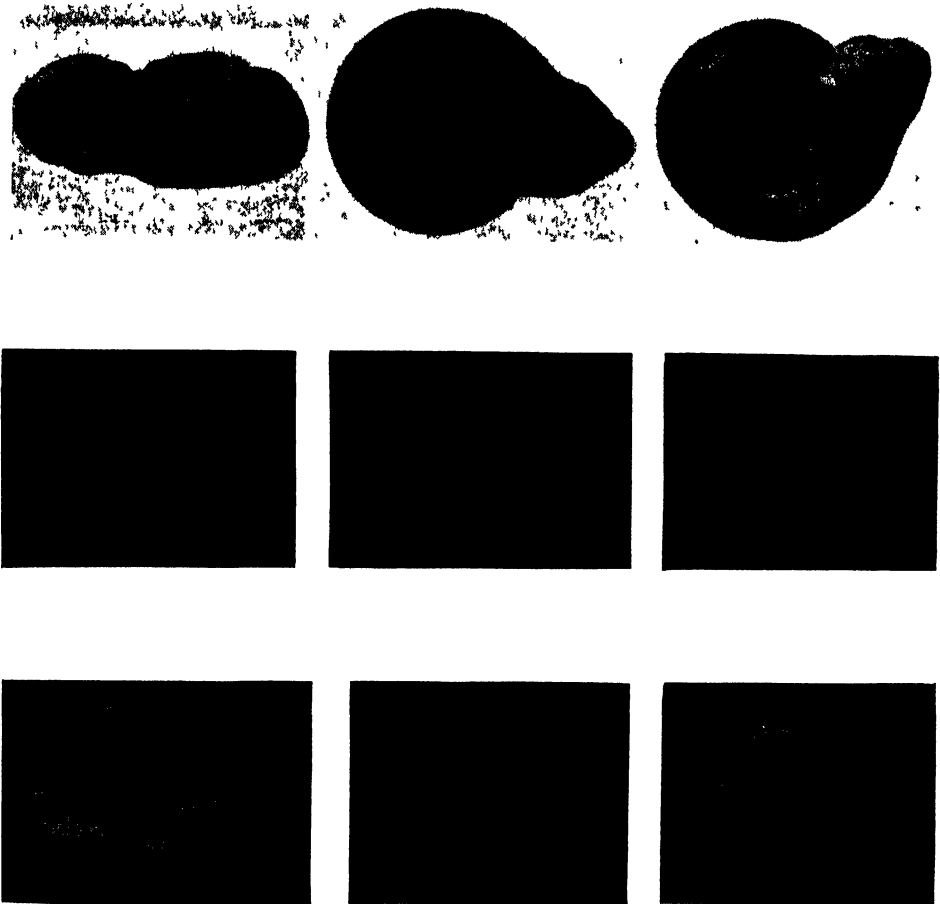


FIGURE 1.

Top Row. *Helisoma campanulatum collinsi* F. C. Baker. Cameron Lake, Lake of the Woods, Ontario. Left and middle figures, paratypes; right figure, holotype. Mus. Nat. Hist., U. of I., No. Z41451, Z41452.

Middle Row. *Helisoma anceps rushi* F. C. Baker. Toad Island, Georgian Bay, Ontario. Left figure, holotype; middle and right figures, paratypes. Mus. Nat. Hist., U. of I., No. Z25259.

Bottom Row. *Helisoma anceps jordanense* (Winslow). Lake Charlevoix, Charlevoix Co., Mich. Topotypes. Mus. Zool., Univ. Mich., No. 61589.

Figures enlarged 2.5 diameters.

This characteristic race of *trivolis* is common in many parts of Ontario. The material in the collection now under study contains many large specimens 25 to 30 mm. in diameter. The race is well figured in the writer's discussion of *Helisoma corpulentum* (4, Plate 5).

*Helisoma pilsbryi infracarinatum* F. C. Baker

Rainy River district: Carp Lake; Knife Lake; Basswood Lake; Shebandowan Lake; Little Knife Lake; Mackenzie Arm and other parts of Kahnipiminanikok Lake; between Saganagons and Kahnipiminanikok Lakes.

The examination of a large series of this form of *Helisoma*, from a wide area of distribution, has convinced the writer that it is a race of *pilsbryi* F. C. Baker. Specimens from Ontario are usually quite characteristic and easily separated from *pilsbryi*, but in many lots there are specimens with a feeble basal carina indicating variation toward *pilsbryi*. Specimens from Rideau River, near Ottawa, Ontario, vary decidedly toward *pilsbryi*. *Infracarinatum* appears to be more widely distributed than *pilsbryi*. Both forms are figured by Baker (4). *Pilsbryi* is now believed to be a distinct species and not a race of *trivolis*, as was stated (4).

*Helisoma corpulentum* (Say)

Rainy River district: Lake La Croix; Basswood River rapids. Thunder Bay district: Northern Light Lake. Kenora district (Lake of the Woods): Rabbit Point, west of Sabaskong Bay; McPherson Island; Kennedy Island. Minnesota: St. Louis Co., Iron Lake.

The finest specimens of this species came from Lake La Croix. These are large (27 mm. in diameter) and have the well-marked sculpture so characteristic of the species. The known records of this species indicate that it is common and widely distributed in western Ontario. See (4) for a discussion of *corpulentum* and its allies. It is interesting to note that one of Say's original localities for this fine species was Lake of the Woods.

*Helisoma corpulentum multicostatum* F. C. Baker

Rainy River district: Carp Lake; Mercutio Lake; Keats Lake; between Saganagons and Kahnipiminanikok Lakes; Kahnipiminanikok Lake. Thunder Bay district: Lac des Mille Lacs swamp. Kenora district (Lake of the Woods): Nestor falls, east of Sabaskong Bay.

Baker and Cahn (6) included this race under *corpulentum*. In the later paper (4) it is separated and figured. *Multicostatum* is as widely distributed as is typical *corpulentum*, often occurring in the same body of water.

*Helisoma whiteavesi* F. C. Baker

Thunder Bay district: Lac des Mille Lacs; Chief Peter Lake. Rainy River district: Athlestone Lake; Crooked Pine Lake; Basswood Lake; Carp Lake; Kashaboiwé Lake.

This fine *Helisoma*, first noted in Lac des Mille Lacs, is more widely distributed than was indicated in (4). The species is at once recognized by its great axial height, flattened spire, and few basal whorls. It is the climax of development of the *corpulentum* group.

*Helisoma campanulatum* (Say)

Rainy River district: Crooked Pine Lake; Carp Lake. Kenora district (Lake of the Woods): Horseshoe Lake, east of Kakagi Lake; McPherson Island; Beggs Lake.

Material apparently referable to typical *campanulatum* occurs in western Ontario. In some lots, as those from Beggs Lake, there is a tendency to vary toward the race called *canadense*. Only the race *canadense* was noted by Baker and Cahn (6).

*Helisoma campanulatum canadense* Baker and Cahn

Thunder Bay district: Northern Light Lake; Cat Lake; Lac des Mille Lacs. Rainy River district: Otter Track Lake; Kashaboiwe Lake; Carp Lake; Brent Lake; Mackenzie Arm and other places in Kahnipiminanikok Lake; lake near Mercutio Lake; rapid water between Saganagons and Kahnipiminanikok Lakes. Kenora district (Lake of the Woods): Rabbit Point, west of Sabaskong Bay; Kennedy and Russell Islands, near Whitefish Bay; McPherson Island.

This small race of *campanulatum* appears to be the dominant form of the species in western Ontario. It is described and figured by Baker and Cahn (6, p. 57). In many lots there is a tendency to vary toward the typical form of *campanulatum*, while in others the small race is quite distinct.

*Helisoma campanulatum collinsi* Var. Nov. (Fig. 1, top row)

A form of *campanulatum*, apparently abundant in the Lake of the Woods region, differs from all other variations of this species in the form of the spire, which is raised and forms a flattened dome, and in the larger number of closely coiled whorls, which total seven. The base shows  $3\frac{1}{2}$  whorls, and the umbilicus is small, round, and deep. The holotype measures: height, 6.0; major diameter, 15.7; lesser diameter, 12.8; aperture height, 5.5; aperture diameter, 4.2 mm. Types from Cameron Lake, northeast of Kakagi Lake (Lake of the Woods); Holotype Z41451, Paratypes Z41452, Museum of Natural History, Univ. of Ill.

This race of *campanulatum* bears some resemblance to *H. multivolvis* (Case) in the raised spire and tightly coiled whorls. The latter, however, has eight whorls, and the base is reamed out much as in *H. campanulatum rudentis* Dall. Also, the spire whorls in *multivolvis* are higher and give a mammiform appearance to the upper surface of the shell.

Besides the type locality, where the variety is very abundant, the new race has been seen from Otterskin and Shingwak Lakes, respectively east and northeast of Kakagi Lake, Lake of the Woods district.

This interesting race of *campanulatum* is dedicated to Dr. W. H. Collins, late Director of the National Museum of Canada. Dr. Collins was greatly interested in the development of the study of Canadian Mollusca.

*Planorbula armigera* (Say)

Thunder Bay district: Lac des Mille Lacs; Wauwiag River. Rainy River district: Snodgrass Lake; Kashaboiwe Lake; Reserve Arm, Lake Kahnipiminanikok. Kenora district (Lake of the Woods): Beggs Lake.

As previously noted (Baker and Cahn, 1931, p. 58), the Canadian material divides into two forms, one with the upper surface of the body whorl sharply carinated and the other with the body whorl rounded. The latter was named variety *palustris* in the paper referred to above, but its distinctness in large series of specimens may be doubted.

*Gyraulus deflectus obliquus* (DeKay)

Thunder Bay district: Wauwiag River. Rainy River district: Mack Lake; lake west of West Lake and north of South Lake. Kenora district (Lake of the Woods): Kennedy Island.

This characteristic race of *deflectus* appears to be widely distributed in western Ontario.

*Gyraulus latestomus* F. C. Baker

Rainy River district: McAree Lake.

This recently described species of *Gyraulus* (2, p. 9) related to but distinct from *G. deflectus obliquus*, is at present known only from the type locality.

*Gyraulus hornensis* F. C. Baker

Rainy River district: Othermans Lake; lake west of West Lake and north of South Lake.

Much of the material listed in (6) is referable to *hornensis* rather than to *arcticus*, which is probably confined to Greenland. The records at hand indicate that *hornensis* has a wide distribution in Canada. See (3) for the description of this species.

FAMILY ANCYLIDAE

*Ferrissia parallela* (Haldeman)

Rainy Lake district: Otter Track Lake.

Common and variable in this lake. As special search must be made for these minute freshwater limpets, the few specimens found by Dr. Cahn in Ontario can scarcely be considered sufficient to indicate the distribution of the group in this area.

## FAMILY PHYSIDAE

*Physa gyrina* Say

Thunder Bay district: Savanne River; Lac des Mille Lacs. Rainy River district: Lake La Croix; Snodgrass Lake; Emerald Lake; Kashaboiwe Lake; Keats Lake; Crystal Lake, north of Pickerel Lake; Reserve Arm of Kahnipinikanikok Lake. Minnesota: St. Louis Co., Iron Lake.

Most of the material from western Ontario is referred to *gyrina* by Dr. Clench. The specimens are small for the most part, scarcely exceeding a half-inch in length. One specimen from Snodgrass Lake is 19 mm. in length, indicating that the species does approach in size the *gyrina* of northern United States. Possibly much of the Ontario material is immature. All specimens have thin shells.

*Physa sayii* Tappan

Thunder Bay district: Mack Lake. Rainy River district: Mercutio Lake; Sark Lake; Emerald Lake. Kenora district (Lake of the Woods): Rabbit Point, northwest of Sabaskong Bay; McPherson Island.

*Physa sayii* is apparently widely distributed in western Ontario. The shell attains a large size (21 mm. in length) and is generally thinner than the shells found farther south.

*Physa warreniana* Lea

Rainy River district: Cub Lake; Sark Lake. Kenora district (Lake of the Woods): Otterskin Lake, east of Kakagi Lake.

The specimens collected by Dr. Cahn are apparently the first of this species to be listed from Ontario. The specimens are large, an individual from Sark Lake measuring 22 mm. in length. Although only beach specimens were collected by Dr. Cahn, the wide distribution of the species would indicate that it is common in western Ontario. Both *sayii* and *warreniana* occurred in Sark Lake.

*Physa integra* Haldeman

Kenora district (Lake of the Woods): McPherson Island.

A single bleached shell apparently referable to this species was found on the beach. (Identification by F. C. Baker.)

*Aplexa hypnorum* (Linn.)

Rainy River district: Mack Lake.

A few specimens of this widely distributed species were collected by Dr. Lindenberg.

## Pulmonata

The pulmonate material listed was collected for the most part by Dr. Lindenberg in the Quetico Provincial Park, Rainy River district, Ontario. A few species were collected by Dr. Cahn. In the list the initials (C) and (L) indicate the collector.

## FAMILY ZONITIDAE

*Retinella binneyana* (Morse)

Thunder Bay district: Wauwiag River (C). Rainy River district: Macle Lake; Agnes Lake; west end, Russell Lake; near Mackenzie Arm of Lake Kahnipiminanikok (L). Kenora district: Kennedy Island (C).

Typical specimens of this northern species of *Retinella*.

*Hawaiiia minuscula* (Binney)

Thunder Bay district: Wauwiag River (C).

Only one specimen collected.

*Striatura milium* (Morse)

Rainy River district: Mackenzie Arm, Lake Kahnipiminanikok (L).

One specimen of this diminutive species was found under a log.

*Zonitoides arboreus* (Say)

Thunder Bay district: Shebandowan Lake; Wauwiag Lake (C). Rainy River district: near Lake Agnes; Mack Lake; west end, Russell Lake; Louis Lake; Mackenzie Arm, Lake Kahnipiminanikok (L). Kenora district: Kennedy Island (C).

This species appears to be as common in Ontario as it is in the United States.

*Euconulus fulvus* (Müller)

Rainy River district: west end, Russell Lake; Mackenzie Arm, Lake Kahnipiminanikok; Mack Lake (L).

Only a few specimens of this cosmopolitan species were collected. They are similar to specimens from Minnesota and Wisconsin.

*Vitrina limpida* Gould

Rainy River district: west end, Russell Lake. Several typical specimens collected by Dr. Lindenberg.

## FAMILY ENDODONTIDAE

*Anguispira alternata* (Say)

Kenora district (Lake of the Woods): Kennedy Island (C). Two shells. Rainy River district: between Louisa and Agnes Lakes (L). One specimen.

*Discus cronkhitei anthonyi* (Pilsbry)

Thunder Bay district: Wauwiag River; Shebandowan Lake (C). Rainy River district: Mack Lake; west end, Russell Lake; near Lake Agnes; near Mackenzie Arm, Lake Kahnipiminanikok (L).

The *anthonyi* from Ontario were found under conditions similar to those under which they live in central United States. The whorls are rounder than in *anthonyi* from some parts of the United States, and in some specimens approach *cronkhitei*.

FAMILY PUPILLIDAE

*Columella edentula* (Drap.)

Several specimens were collected from near the shore of Mack Lake, Rainy River district (L).

*Vertigo modesta* (Say)

Rainy River district: Mack Lake; near Mackenzie Arm of Lake Kahnipiminanikok; west end, Russell Lake (L).

One specimen from the first, two specimens from the second, and one specimen from the third locality.

*Zoogenites harpa* (Say)

Rainy River district: Mack Lake; west end, Russell Lake; near Mackenzie Arm, Lake Kahnipiminanikok (L).

This peculiar little mollusc appears to be common in Ontario. The specimens were found under logs near the camp ground.

FAMILY STROBILOPSIDAE

*Strobilops labyrinthica* (Say)

Rainy River district: Mack Lake, near Agnes Lake; west end, Russell Lake (L).

The specimens were found under logs and loose bark. Apparently common.

FAMILY COCHLICOPIDAE

*Cochlicopa lubrica* (Müller)

Rainy River district: Mack Lake (L).

Several specimens of this widely distributed species were collected.

FAMILY SUCCINEIDAE

*Succinea ovalis* Say

Kenora district: McPherson Island (Lake of the Woods) (C). One half-grown specimen. Rainy River district: near Agnes Lake (L). Two specimens.

*Succinea grosvenori* Lea

Thunder Bay district: Lac des Mille Lacs (C).

Two specimens were collected from debris.

*Succinea retusa* Lea

Thunder Bay district: Lac des Mille Lacs. Rainy River district: shore of Lake Kahnipimianikok. Kenora district (Lake of the Woods): McPherson Island (C).

A few specimens were found on the shore of the lakes mentioned. *Retusa* and *grosvenori* were identified by Dr. Harold A. Rehder, of the United States National Museum.

## FAMILY PHILOMYCIDAE

*Pallifera dorsalis* (Binney)

Rainy River district: Mack Lake (L).

One specimen of this slug was collected. It is like the form found in Michigan.

## FAMILY LIMACIDAE

*Deroceras* cf. *gracile* Raf. (*Agriolimax campestris* Binney)

Rainy River district: Louisa Lake; west end, Russell Lake; Agnes Lake (L). A small slug, believed to be this species, was collected from logs near camps.

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# PATHOGENICITY OF *BEAUVERIA BASSIANA* (BALS.) VUILL. ON COLORADO POTATO BEETLE LARVAE<sup>1</sup>

By M. I. TIMONIN<sup>2</sup>

## Abstract

*Beauveria Bassiana* (Bals.) Vuill. isolated from infected Colorado potato beetle larvae is shown to be a pathogen to this host. The experiments indicated that some of the infected larvae may pass through the larval stage, but owing to further mycelial growth finally succumbed in the pupal stage. The fungus does not attack the eggs, but infected young larvae were found dying on the fourth day after hatching, when the eggs had been dusted with *B. Bassiana* spores. The mycelial growth on the infected larvae is more rapid in the soil than above ground.

## Introduction

In September 1935, dead larvae of the Colorado potato beetle (*Leptinotarsa decemlineata* Say) were collected in a potato field at Fredericton, New Brunswick, and forwarded for examination to the Division of Botany by Mr. R. P. Gorham, Dominion Entomological Laboratory, Fredericton, N.B. The larvae were covered with soil particles, but a thin white mycelial growth, incrusting with conidial heads, dotted the surface of the larvae. The fungus was identified as a *Beauveria* sp. and was isolated in pure culture.

In 1935, Poisson and Patay (2) described a new species of the genus *Beauveria* pathogenic to Colorado potato beetle larvae and named it *Beauveria doryphorae*. Their description of the fungus may be summarized as follows: Culture white in colour, dense, velvety, forming a mycelial turf with a chalky appearance due to the abundant production of conidia; conidia perceptibly oval, 1.8 to 2.0  $\mu$  in diameter, and the phialides bearing them ventricose; phialides and conidia forming an aggregation about 30  $\mu$  in diameter; potato media not coloured red by the fungus.

The Canadian fungus, grown on potato agar with two per cent dextrose, is at first white, becoming light cream in colour later. Mycelial growth consists of a cottony or fluffy mass of hyphae, and frequently spreads over the glass in strands, filling the test-tube almost entirely within 10 to 15 days of incubation. Prothialides are ovoid to globose in whorls, bearing one or more, frequently two, phialides. Phialides are oval or flask-shaped with a long thread-like beak of zigzag shape. Conidia are globose or globoid, about 2.0 to 3.5  $\mu$  long and 2.0 to 3.0  $\mu$  wide. The reverse colour of the colony is cream buff and the colour of the medium remains unchanged. The conidia and conidia-bearing organs closely resemble those of *Beauveria Bassiana* isolated by the author from European corn borer larvae, but the latter isolate differed greatly in gross appearance owing to the abundant production of conidia in place of mycelium.

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If the above descriptions are compared, it is evident that the Canadian isolation differs from the European by the type of growth and the colour of mycelium, as well as in the shape and size of the conidia. A culture of the Canadian fungus was sent to Dr. C. Petch, who identified it as *Beauveria Bassiana* (Bals.) Vuill.

The purpose of this investigation was to determine whether this fungus is a pathogen of Colorado potato beetle larvae.

### Material and Methods

With a view to securing sufficient spores of *Beauveria Bassiana* for field experiments, the fungus was grown on soybean mash in Petri dishes. The method used in the preparation of the mash, and propagation of the fungus was the same as that already described in a field experiment with *Beauveria Bassiana* (3). After four to five days of incubation, the fungus developed luxurious mycelial growth, but did not form spores as freely as isolates of *B. Bassiana* from corn borers on the same medium.

In order to test the pathogenicity of *Beauveria Bassiana*, several potato hills, naturally infested with Colorado potato beetle larvae, were selected in the experimental potato plots and caged with cheese cloth.

The larvae were dusted with the conidia by dipping a small camel's-hair brush into a culture of the *Beauveria* and gently tapping it while it was held over the larvae.

### Pathogenicity of *Beauveria Bassiana*

On July 15th, 1936, four potato hills were caged with cheese cloth, the larvae counted and in three cages they were dusted with *Beauveria* spores. The fourth was kept as a check. The larvae at that time were in the prepupal or in the last larval instar stage. The results of this experiment are summarized in Table I.

TABLE I  
PATHOGENICITY OF *BEAUVERIA BASSIANA* ON COLORADO POTATO BEETLE LARVAE

Cage No.	No. of larvae dusted	No. of larvae infected 3 days after dusting	No. of dead larvae found 6 days after dusting	No. of dead larvae found in the soil 14 days after dusting	No. of pupae found in the soil	No. of pupae with brown marks and mycelial growth	No. of pupae with healthy appearance	Per cent of survival
1	21	18	7	8	5	3	2	9.5
2	19	19	5	12	2	2	0	0
3	25	21	9	8	8	4	4	16.0
4	20 untreated (check)	None	None	1	19	None	19	95.0

On the third day after inoculation, brown to dark brown lesions were observed under the epidermis of the infected larvae (Fig 1), while at the same time uninoculated larvae in the control cage remained normal. Larvae

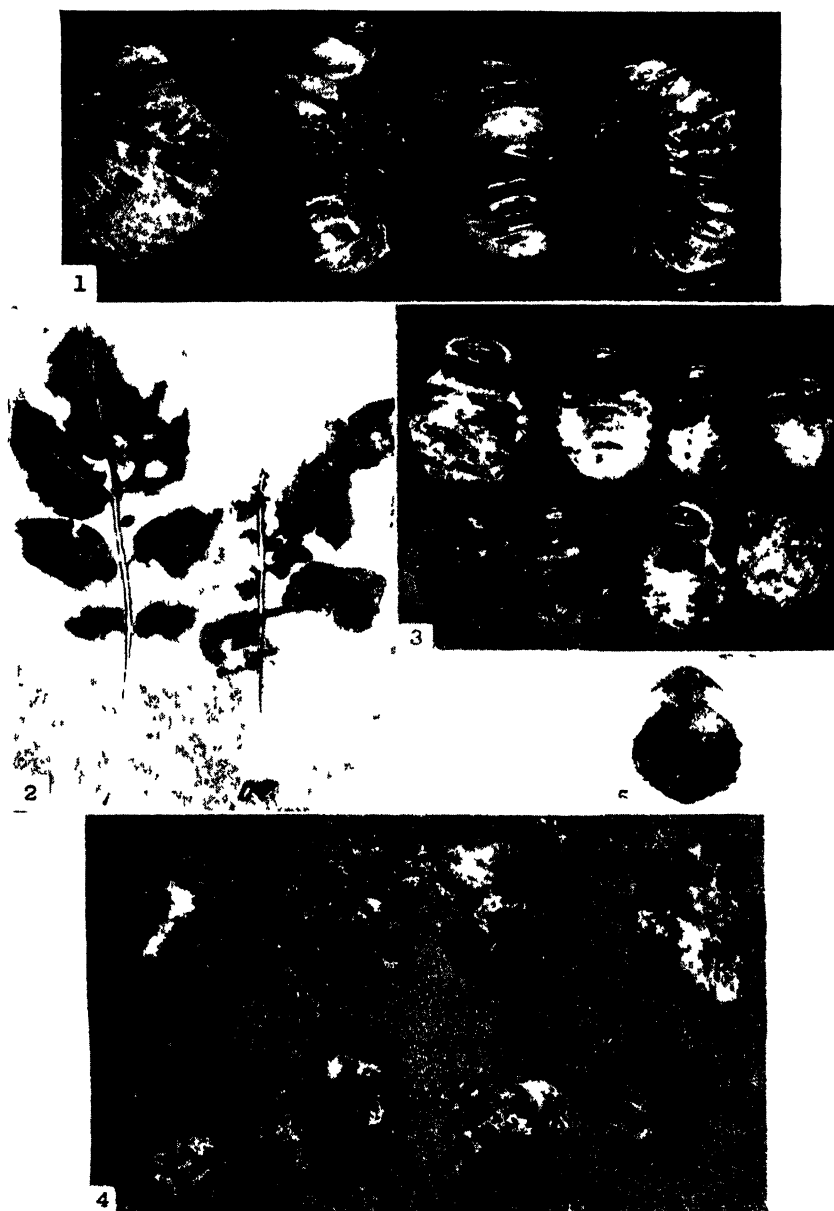


FIG. 1. Larvae four days after dusting. Note the dark brown lesions of infection.  $\times 3\frac{1}{2}$ . FIG. 2. Mummified larvae attached to the leaves  $\times 3\frac{1}{2}$ . FIG. 3. Mummified larvae found on the ground.  $\times 3\frac{1}{2}$ . FIG. 4. Mummified larvae, surrounded with soil particles, from soil under dusted plants.  $\times 2$ . FIG. 5. Pupa with dark lesions of infection.  $\times 2$ .

bearing such lesions were somewhat less active and did not feed as freely as the larvae in the control cage. When the lesions reached a diameter of 2 to 3 mm. (Fig. 1, second larvae from the left) the larvae became practically motionless and died shortly afterwards. The interiors of the dead larvae were completely filled with mycelium and some mycelial growth developed on the surfaces of the larvae.

This observation is somewhat similar to that reported by Boczkowska (1). Studying immunity of the larvae of *Galleria mellonella* L. to entomogenous fungi, she observed that caterpillars infected with the entomogenous fungi often developed black spots under the chitin. These black spots were found to contain agglomerations of leucocytes. She also noticed that larvae covered with lesions 1 to 2 mm. in diameter always succumb, whereas those with delicate spots sometimes survive and pass through metamorphosis.

Only a few dead larvae remained attached to the leaves of the potato plants (Fig. 2); the majority of the larvae fell to the ground. Dead larvae which were collected on the ground were covered with more surface mycelial growth than those which remained attached to the leaves (Fig. 3).

Metamorphosis of the larvae occurs below ground. Probably because conditions were more suitable for the fungus growth, many of the infected larvae that entered the soil became mummified after three to four days. The mycelium spread in all directions through the soil particles and bound them together, completely surrounding the larvae in a layer of soil which formed a soil ball 1 to 1.5 cm. in diameter incrustated with white mycelial threads (Fig. 4).

Some of the larvae successfully passed into the pupal stage, but the dark infected areas could still be seen on the epidermis of the pupae (Fig. 5).

Two flower pots were filled with greenhouse soil and in one four healthy, and in the other four infected, pupae were buried about 2 in. deep. The pots were kept in the greenhouse, and within four days of incubation four adults emerged from the healthy pupae. The second flower pot, containing the infected pupae, was examined on the fourth day of incubation, when all four pupae were found to be mummified and to have the same appearance as the larvae in Fig. 4.

The daily temperatures, humidity of the air, and rainfall during the period of this experiment, have been obtained through the courtesy of the meteorological station at the Central Experimental Farm, Ottawa. Taking into consideration these data, we may conclude that the climatic conditions prevailing were favourable to the fungus growth. The highest temperature during the experiment was 83° F., which is around the optimum for fungus growth; the humidity of the air, according to the two daily readings, was sufficient for mycelial growth, and the few rain showers kept the soil sufficiently moist for the rapid growth of the fungus.

This experiment was repeated several times when larvae and potato plants were dusted with *Beauveria* spores; but plants were not caged in cheese cloth,

and it was impossible to estimate the number of larvae actually involved in the experiment, owing to the fact that larvae were continually emerging from the newly laid eggs. Nevertheless, observations indicated that the fungus does not attack the eggs. However, infected young larvae were found dying on the fourth day after hatching, when the eggs were dusted. It would therefore appear that at any stage of their development the larvae are susceptible to *Beauveria Bassiana* isolated from this host.

### Acknowledgments

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## SOME BLOOD CHEMICAL CHANGES DURING RECOVERY FROM EXHAUSTIVE MUSCULAR EXERCISE<sup>1</sup>

BY HANS SELYE<sup>2</sup>

### Abstract

Experiments in the rat indicate that following a period of intense muscular exercise, marked hyperchloremia develops and is maintained for several days following discontinuation of the exercise. Simultaneously, the red cell concentration decreases and the haematocrit values remain at approximately the same low level at least during the first 72 hr. of the recovery period.

The blood sugar values, which decrease under the influence of muscular exercise, show a marked secondary rise even if the animals are fasted throughout the recovery period.

### Introduction

In the course of our experiments on the effect of muscular exercise on the chemical composition of the blood, we noted that a marked increase in blood chlorides and a decrease in blood sugar are characteristic accompaniments of extreme muscular fatigue (3, 4). Haematological studies showed furthermore that after a single period of excessive muscular exercise, the red cell count may decrease considerably and remain low for over 72 hr. (1). It appeared of interest to establish how long the blood chemical changes elicited by muscular exercise would persist and what changes are characteristic of the recovery period.

### Methods

Six-months-old female "hooded" rats were used for all the experiments reported in the communication. Their body weights varied between 140 and 224 gm. All experimental animals were forced to perform muscular exercise during four one-hour periods within 24 hr. For this purpose they were placed in drum cages having a diameter of 12 in. and revolving at a speed of 18 to 22 r.p.m. During the exercise period all animals were fasted, because previous experiments have shown that the amount of food ingested may have a considerable influence on the response to muscular exercise, and during the exercise period the amount of food voluntarily taken shows marked individual variations. After the 24 hr. of exercise, the rats were divided into two groups, one of which was fed and the other starved. As fasting in itself influences the haematocrit, blood sugar, and blood chloride values, it seemed advisable to run a special set of not exercised, fasting controls every day, to show the effect of starvation by itself.

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The blood sugar determinations were performed with the Schaffer-Hartmann and Somogyi method. The chlorides were determined in whole blood with the Van Slyke method.

### Experimental Results

The results of the experiments are summarized in Fig. 1. Each point on the curves represents the average of three experimental animals killed at the corresponding time. After the completion of the 24-hr. fasting period, the curves representing the exercised animals divide in two, because half of the animals received food during the recovery period, while the other half was fasted. Points on the curves corresponding to the time at which a group of three animals was killed for determinations are marked by squares in the case of the not exercised fasted controls, by dots in the case of the exercised fasted animals, and by crosses in the case of the exercised fed animals. The blood chlorides are expressed as milligrams of sodium chloride per 100 cc. of whole blood and the blood sugars also in milligrams per 100 cc.

The curves in the upper part of Fig. 1 show the average weight loss in grams per rat. It will be seen that although the exercised fasted animals lost a little more weight than the fasted controls, the difference is not very marked. The exercised fed animals, on the other hand, actually gained weight at the end of the experiment so that the curve representing this group declines sharply after the 32nd hr., that is to say, beginning at about 8 hr. after the last exercise period and the administration of food. Between the 72nd and the 96th hr. of the experiment, the curve declines below the zero level of the diagram, since at this time the weight of these animals rose above the initial level.

From the curves representing blood sugar and blood chloride values in the controls, it will be seen that both show a more or less regular and gradual fall, which becomes quite marked at the 96th hr. On the other hand, the blood sugar values in the exercised animals are extremely low at the end of the first 24 hr., but from then on they gradually rise to a level in the fasted animals as high as that of the fed, untreated, normal controls (represented by the point on the ordinate from which all these curves begin). The exercised animals that received food during the recovery period show an actual increase over the initial value at this time. That the initial hypoglycemia caused by muscular exercise may be followed by a secondary hyperglycemic phase even in fasting animals confirms previous observations (5). This is probably due to the fact that exhaustive muscular work elicits a severe "alarm" reaction, characterized by an initial hypoglycemia followed by hyperglycemia. The observation that fasting in itself may elicit a marked "alarm" reaction in thyro-parathyroidectomized rats (2) may explain furthermore why rats deprived of their thyro-parathyroid apparatus respond to continued fasting with an initial hypoglycemia followed by a secondary hyperglycemic phase (3).

The blood chlorides are above normal at the end of the 24-hr. exercise period and remain at this hyperchloremic level for at least 72 hr. from the

beginning of the experiment. There is no constant and significant difference in this respect between the fed and the fasted animals. Since these figures correspond to whole blood chlorides, it would be conceivable that the increase in chloride values is merely due to the decrease in haematocrit values, because

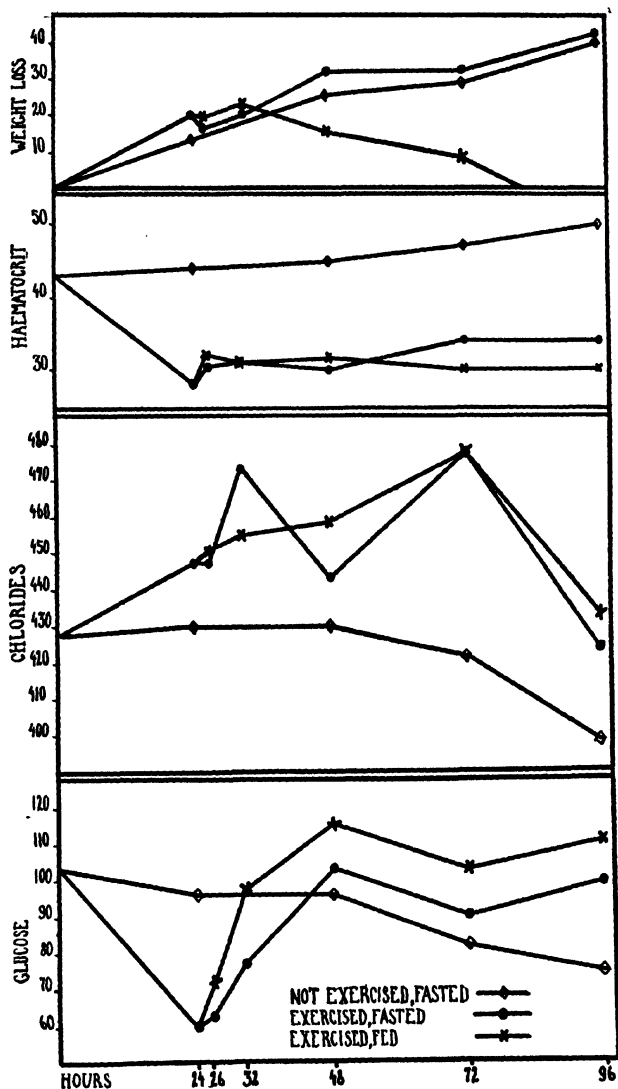


FIG. 1

blood corpuscles are poorer in chlorides than the plasma; consequently, an increase in the relative volume of plasma would necessarily increase the whole blood chloride values unless compensated for by a shift of chlorides from

plasma into tissues. However, Fig. 1 indicates quite clearly that no strict parallelism exists between blood chloride and haematocrit values. For instance, at 72 hr. the blood chlorides are high both in the fed and the fasted exercised animals, while at 96 hr. the chloride concentration of the blood returned to normal in both these groups. On the other hand, the haematocrit values did not change either in the fed or the fasted exercised group at these times.

We must conclude, therefore, that although the rise in blood chlorides is accompanied by, and may partly be due to, a decrease in the red cell concentration, it cannot be regarded simply as a result of the latter. Towards the end of the experiment, the haematocrit values are somewhat higher in the fasted than in the fed group, but this may at least partly be due to the increase in the red cell concentration that is a usual accompaniment of prolonged starvation. In any case, it seems quite evident from the diagrams that the haematocrit values remain at approximately the same low level during the entire length of the 72-hr. recovery period, although the blood chlorides and the body weight return to normal towards the end of the observation period.

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## THE SECRETION OF CRYSTALLOIDS AND PROTEIN MATERIAL BY THE PANCREAS IN RESPONSE TO SECRETIN ADMINISTRATION<sup>1</sup>

By S. A. KOMAROV<sup>2</sup>, G. O. LANGSTROTH<sup>3</sup>, AND D. R. McRAE<sup>3</sup>

### Abstract

The concentrations of sodium, potassium, calcium, chloride, bicarbonate, and protein and non-protein nitrogen were determined in series of samples of pancreatic juice secreted by dogs in response to (a) constant rate of administration of secretin, (b) varied rate of administration of secretin, and (c) interrupted administration of secretin (rest period, 2 hr.). Spectroscopic as well as chemical methods were used in the analysis of the samples.

The data indicate, that the membranes of the pancreatic gland offer little resistance to the passage of simple inorganic ions. The compensatory relation between the bicarbonate and chloride concentrations of the secretion is interpreted on the basis of (a) formation of at least part of the bicarbonate within the secretory cells, and (b) the action of membrane forces, probably of an electrical nature. Absorption spectrum studies indicate either (a) that only one type of absorbing protein is present in the secretion, or (b) that if several types are present, they are always secreted in constant proportions whether they are enzymatically active or not. The interpretation of the protein nitrogen and certain other data are given in a subsequent paper.

A study of the dependence of the composition of a glandular secretion on the conditions of stimulation and on the previous experimental history of the gland may be expected to yield some insight into the nature of the secretory processes. This expectation has been realized for the submaxillary gland (11). The present article describes a study of the secretion of crystalloids and protein material by the pancreas of the dog under various conditions of secretin administration. Three important features of the technique employed in these investigations may be noted here. (a) Only comparatively small samples of secretion were taken from the gland. The interpretation of differences in composition is thereby simplified, since relatively small changes occur in the intra-glandular conditions during short periods of secretory activity. Each of the consecutive samples comprising an experiment was analyzed for its major constituents, as any detailed interpretation of composition differences between widely separated samples requires some knowledge of the behaviour of the gland during the intervening period. These considerations are of the greatest importance in studies of the secretion of protein material. (b) The use of quantitative spectroscopic methods of analysis from both emission and absorption spectra, combined with chemical methods, permitted relatively complete analyses of the comparatively small samples to be made. In addition, absorption spectrum measurements yielded information not easily obtained by other means. (c) A mathematical treatment of the synthesis and secretion of protein material aided considerably in designing experiments to

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show certain characteristic features of the glandular behaviour. This treatment and the interpretation of much of the data of the present article are given in the paper that follows.

The older investigations (reviewed by Roseman (15), Babkin (4, p. 462), and Ball (6, 7) ) have indicated that the external secretion of the pancreas originates in a single type of secretory cell, and that it may be evoked by various stimuli. The composition of the secretion varies with the type of stimulus applied. It has been suggested (12) that secretion of protein material is governed solely by the action of the vagus nerves and that secretin is responsible for the secretion of water and crystalloids. This view cannot easily be reconciled with the experimental fact that large quantities of protein material are present in the secretion evoked by secretin even when the action of the vagi is eliminated by section or by administration of large doses of atropine (4, p. 601; also the data of the present article). Furthermore Hammarsten, Ågren and Lagerlöf (10), investigating the effects of injection of purified (but not crystalline) secretin in man, have recently obtained data which they interpret as proving that "secretin stimulates a real time-rate production of enzymes from the pancreatic gland." It therefore appears that secretin itself must be considered to control a mechanism that results in the secretion of protein material as well as of crystalloids and water. For reasons to be stated later the present data are believed to be descriptive of this mechanism.

The more recent investigations (6, 7) have been mainly concerned with the effect of intravenous injections of acids, bases and salts, on the composition of the pancreatic secretion obtained from anaesthetized dogs under secretin administration. There has been no systematic study of the changes in the concentrations and outputs of the inorganic, protein, and non-protein components of the secretion with changes in glandular activity under intravenous injection of secretin.

### Experimental Procedure

(a) *Physiological technique.* Dogs weighing from 5.8 to 17.5 kg. were used throughout the research. They were fasted for 24 hr., and anaesthetized with nembutal. After tracheotomy the vagi were cut in the neck, the abdomen was opened, the small pancreatic duct tied, and the main pancreatic duct cannulated. In order to prevent passage into the intestine of any gastric juice that might be secreted, the stomach was separated from the duodenum by a ligature around the pyloric part of the mucous membrane. The passage of bile into the intestine was prevented by cannulating the bile duct. Artificial respiration was used only in a few cases, when respiration ceased, probably because of an excess of the anaesthetic. A known amount of a secretin solution was introduced every 5 min. into the femoral vein, and the pancreatic juice secreted was collected in graduated test-tubes kept in a beaker containing ice. For the most part the secretion was collected at intervals of 30 min.

The same preparation of secretin, obtained by Still's (17) method from the duodenum and upper jejunum of dogs, was used throughout the work. It corresponded to the preparation designated by Still as "crude" secretin, but was purified further by dissolving in *N*/50 sulphuric acid and precipitating with 10% trichloroacetic acid. The precipitate was extracted with acetone and ether, dried, and preserved in a vacuum desiccator over sulphuric acid in the presence of soda lime. The potency of the preparation did not change to any marked extent during the six months required for the experimental work. The preparation was free from vaso-depressor substances and had only a slight insulin-like effect on the blood sugar.

(b) *Analysis of the samples.* Total solids and ash were determined in the usual manner, and chlorides by Wilson and Ball's (19) modification of Van Slyke's method. Acid combining power was determined by heating 1 cc. of pancreatic juice with an excess of *N*/50 sulphuric acid (5 or 10 cc.) in a boiling water bath for 10 min., and back titrating with *N*/50 sodium hydroxide to methyl red. It was established in a special set of experiments that the figures so obtained for the acid combining power were virtually identical with those for the carbon dioxide combining power, or alkali reserve, as determined by the volumetric method of Van Slyke and Cullen (13, p. 251). Hence the acid combining power is regarded as representing the bicarbonate content of the secretion. In the determination of non-protein nitrogen, the protein-free filtrates were obtained by precipitating the protein material with five volumes of 9% solution of trichloroacetic acid immediately after the sample was collected. The nitrogen in these filtrates, as well as the total nitrogen in the secretion, was determined by the micro-Kjeldahl method (14, p. 120).

The absorption spectrum of the secretion consisted only of the so-called protein band near  $\lambda 2800$ , and end absorption near  $\lambda 2500$ . Change in the rate of secretin administration merely altered the strength of the  $\lambda 2800$  absorption. This was measured by a method employing rotating step-sectors and requiring less than 0.3 cc. of secretion for a determination. Knowing the strength of the absorption, an "effective"  $\epsilon c$  value could be calculated from the ordinary absorption law ( $\epsilon$  = extinction coefficient,  $c$  = concentration). In order to give these values some meaning, they were correlated with protein nitrogen data, as will appear later. The secretion was collected and stored on ice for the short time between taking the sample and measuring the absorption. Subsidiary tests showed that under these conditions no appreciable aging or auto-digestive effects occurred.

Sodium, potassium, and calcium were determined by a quantitative spectroscopic method (9).

## Results

Results for typical experiments are given in Tables I, II, and III. In the experiment of Table I, the rate of administration of secretin was kept constant throughout, and in that of Table II it was varied. In the experiment of Table III, the administration of secretin was stopped in the middle of the experiment and resumed after a period of 2 hr.

TABLE I  
Experiment 5: Dog, male, 15.4 kg. Constant rate of secretin administration.

Sample No.	Vol.†	Na	K	Ca	Cl	ACP	NPN	PN	ec	Solids		
										Total	Org.	Ash
1	8.7	—	—	—	34.8	133	19.3	341	36.0	3.18	2.22	0.96
2	10.0	146	3.3	1.2	30.0	139	22.7	227	24.5	2.46	1.51	0.95
3	10.0	—	—	—	33.2	136	18.5	185	18.7	2.16	1.22	0.94
4	10.0	131	3.3	0.9	38.0	131	18.5	160	15.8	1.99	1.05	0.94
5	10.5	—	—	—	44.8	124	18.5	134	15.6	1.90	0.95	0.95
6	10.3	131	3.1	1.0	48.8	121	18.5	118	13.1	1.81	0.85	0.96
7	10.3	—	—	—	54.4	115	18.5	110	13.1	1.75	0.79	0.96
8	10.0	139	3.3	1.1	58.0	111	18.5	108	13.1	1.72	0.76	0.96

Rate of injection of secretin was 6.1 mg. per 5 min. throughout. The strength of the secretin solution was 2.04 mg. per cc. of secretin in 0.9% sodium chloride plus 0.024 cc. N sulphuric acid. All samples were 30-min. samples.

† Volume (cc.) of pancreatic juice collected in 30 min.

TABLE II  
Experiment 6: Dog, male, 7.5 kg. Rate of secretin administration varied.

Sample No.	Inj.*	Vol.	Na	K	Ca	Cl	ACP	NPN	PN	ec	Solids		
											Total	Org.	Ash
1	6.0	8.5	—	—	—	86.8	76.4	23.5	164.0	—	2.15	1.22	0.93
2	6.0	10.7	—	—	—	82.0	80.4	21.0	118.0	14.1	1.83	0.89	0.94
3	6.0	10.4	—	—	—	81.6	80.8	21.0	96.0	—	1.69	0.76	0.93
4	6.0	10.6	—	—	—	82.4	81.2	17.6	83.7	—	1.62	0.67	0.95
5	6.0	10.6	143	3.9	3.1	83.2	80.4	17.6	84.3	10.2	1.59	0.64	0.95
6†	1.5	2.0	—	—	—	(93.0)	(67.0)	17.6	55.2	—	—	—	—
7	1.5	4.3	139	3.9	3.3	99.2	58.8	17.6	74.8	8.85	1.50	0.59	0.91
8	1.5	3.7											
9†	6.0	3.4	—	—	—	(93.0)	67.6	19.6	71.7	—	—	—	—
10	6.0	11.6	—	—	—	86.4	74.8	17.9	107.0	—	1.76	0.82	0.94
11	6.0	12.2	—	—	—	86.8	77.6	17.9	96.9	11.4	1.70	0.75	0.95
12†	1.5	3.0	—	—	—	(93.0)	66.8	17.9	72.8	—	—	—	—
13	1.5	4.5	—	—	—	105.0	54.8	19.6	77.3	9.2	1.58	0.65	0.93
14	1.5	3.0											
15†	6.0	3.5	—	—	—	93.6	67.6	19.0	124.0	—	—	—	—
16	6.0	12.2	—	—	—	89.2	74.4	17.9	96.9	11.4	1.74	0.78	0.96
17	6.0	13.0	—	—	—	88.4	75.6	19.6	81.2	—	1.59	0.65	0.94
18	6.0	13.5	113	3.1	3.0	86.4	76.0	17.9	74.5	9.2	1.55	0.62	0.93
19	6.0	13.2	—	—	—	87.2	75.2	17.9	68.9	—	1.50	0.56	0.94
20†	1.0	3.3	—	—	—	—	—	19.6	43.7	—	—	—	—
21	1.0	3.7	143	3.3	3.1	106.0	50.0	19.6	55.4	6.7	1.40	0.48	0.92
22	1.0	2.8											
23	4.0	10.5	—	—	—	88.2	72.0	17.9	67.8	—	1.52	0.57	0.95
24	4.0	9.5	122	3.5	3.7	90.0	72.8	21.8	62.2	8.4	1.47	0.53	0.94
25**	4.0	10.8	170	3.7	3.7	88.4	74.4	20.2	58.8	7.6	1.49	0.54	0.95
26**	4.0	11.0	—	—	—	90.0	76.4	19.6	57.7	—	1.44	0.50	0.94

\* The strengths of the secretin solutions injected were 2 mg. per cc. secretin (Nos. 1-5, 9-11, 15-19, 23-26), and 0.5 mg. per cc. secretin (Nos. 6-8, 12-14, 20-22), in saline plus N/50 sulphuric acid.

† These samples were taken to clear the ducts of previous secretin; Nos. 6, 9, and 15 were 10-min. samples; Nos. 12 and 20 were 15-min. samples; remainder were 30-min. samples.

\*\* Atropine was injected before No. 25, and again during No. 26.

TABLE III

Experiment 7: Dog, female, 17.5 kg. Secretin administration stopped in the middle of the experiment, and resumed again after a period of 2 hr.

Sample No.	Inj.*	Vol.	Cl	ACP	NPN	PN	ec
1	6.0	4.7	68.0	101	22.7	410	—
2**	6.0	8.8	67.6	104	21.8	297	—
3	8.0	6.4	65.6	106	21.8	174	22.4
4	8.0	6.0	64.8	106	23.5	165	—
5	8.0	6.2	63.6	106	23.5	156	—
6	8.0	6.3	64.8	106	25.2	150	17.0
7†	0.0	0.8	—	—	—	—	—
8†	0.0	0.2	—	—	—	—	—
Rest period, 2 hr.							
9	8.0	5.2	86.4	82.4	31.1	355	(35)
10	8.0	5.9	74.0	98.0	31.1	246	—
11	8.0	5.9	72.8	100	31.1	232	—
12	8.0	6.1	69.6	102	31.1	187	—
13	8.0	6.4	71.2	101	31.1	172	19.4

\* The strength of the secretin solution injected was 2 mg. per cc. secretin in saline plus N/50 sulphuric acid.

\*\* Sample No. 2 was a 65-min. sample.

† Samples Nos. 7 and 8 were 5-min. samples taken to ascertain how the rate of flow fell off on stopping the secretin administration. All samples except Nos. 2, 7, and 8 were 30-min. samples.

In these tables, "Inj." denotes the rate of administration of secretin in mg. per 5 min., and "Vol." the volume (cc.) of pancreatic juice collected in each 30-min. period, except where noted. The sodium (Na), potassium (K), calcium (Ca), and chloride (Cl) concentrations and the acid combining power (ACP) are given in milliequivalents per litre, protein and non-protein nitrogen (PN and NPN) in mg. per cent, and total solids, organic matter, and ash in gm. per cent. The *ec* values, calculated per cm. path, are purely relative.

A small sample of secretion was taken immediately after each change in the rate of secretin administration in order to clear the ducts of secretion resulting from the previous stimulation. Such samples (e.g., Nos. 6, 9, 12, 15, and 20 of Table II) may be expected to have a composition intermediate between that of the preceding and that of the following sample, and are of little value in the interpretation of the results.

The following remarks may be made concerning the above data, which are representative of those of other similar experiments.

(a) *The composition of the pancreatic juice secreted under a constant rate of secretin administration (Table I).* With the exception of the first sample, the rate of secretion of water remained essentially constant throughout the experiment (4 hr.). The concentrations of the investigated metals (sodium, potassium, and calcium) also remained constant. A gradual decrease in the acid combining power during the experiment was compensated for by an increase in the chloride concentration, the sum of the two in milliequivalents per litre remaining constant. The concentration of ash, which is largely

determined by the above-mentioned substances, remained the same throughout the experiment. The concentration of protein nitrogen gradually decreased, the rate of decrease being greatest for the first few samples. The non-protein nitrogen concentration remained unchanged; in some experiments, however, it increased slightly toward the end—*e.g.*, in Experiment 3 (not given here) it rose from 30.8 to 39.2 mg. per cent in  $4\frac{1}{2}$  hr.

It was thought that the gradual decrease in the acid combining power and the compensatory relation with the chloride concentration might be due in part to loss of bicarbonates from the system during the experiment, and to the fact that secretin was injected in acid solution. A special experiment in which amounts of sodium bicarbonate equivalent to the bicarbonate loss in the secretion were injected intravenously every half-hour, however, gave results similar to those of experiments in which no replacement was made. The acid combining power fell from 129 to 102, and the chloride concentration rose from 40.7 to 66.2 milliequivalents per litre during the experiment (5 hr.). The results are given in Table IV. The decrease in acid combining power might at first sight be thought to be related to the decrease that occurs in the protein nitrogen concentration. This view is not tenable, however, in the light of such data as given in Table III, which show that the acid combining power of the secretion is not increased after an appreciable period of rest, although a considerable increase takes place in the amount of protein material secreted.

TABLE IV

Experiment 14: Dog, female, 6.5 kg. Constant rate of administration of secretin; bicarbonate lost in secretion replaced by equivalent injections of sodium bicarbonate.

Sample No.	Time	Vol.	Cl	ACP	NPN	PN	NaHCO <sub>3</sub> injections		
							Time, p.m.	Vol., cc.	Concent., m. eq./l.
	p.m.								
1	2:00 - 2:10	2.5	—	—	18.9	357.0	—	—	—
2	2:10 - 2:40	7.2	40.7	129	20.2	157.0	—	—	—
3	2:40 - 3:10	7.3	44.0	124	20.2	133.0	—	—	—
4	3:10 - 3:40	7.7	46.9	122	17.6	115.0	3:17	7.3	130
5	3:40 - 4:10	8.7	51.2	118	16.0	102.0	3:50	7.5	125
6	4:10 - 4:40	9.4	54.6	113	17.6	86.6	4:16	8.7	122
7	4:40 - 5:10	9.8	57.2	111	17.6	77.6	4:49	9.4	118
8	5:10 - 5:40	10.3	61.1	107	17.6	72.3	5:19	9.8	113
9	5:40 - 6:10	10.5	62.4	106	18.5	66.6	5:48	10.3	111
10	6:10 - 6:40	10.6	63.9	104	17.6	64.7	6:18	10.5	107
11	6:40 - 7:10	10.5	66.2	102	20.2	61.0	6:47	10.6	106

*Rate of injection of secretin was 12 mg. per 5 min. throughout. The strength of the secretin solution was 4 mg. per cc. of secretin in saline containing N/200 sulphuric acid.*

(b) *The effect of variations in the rate of administration of secretin on the composition of the pancreatic juice (Table II).* In Experiment 6 and similar experiments, secretin was injected every 5 min., but the amount injected was varied at the indicated intervals. In the earlier part of the experiment this

was done by varying the strength of the secretin solution, but in the latter part the volume injected was also varied.

The data show that a decrease in the rate of secretin administration results in a decreased rate of flow of water from the gland. It also results in a decrease in the acid combining power and an increase in the chloride concentration of the secretion, the changes being such that the sum of these two factors in milliequivalents per litre remains practically unchanged. The concentrations of the investigated metals, the ash and the non-protein nitrogen are little affected by changes in the rate of secretin administration.

There are wide variations in the behaviour of the protein nitrogen *concentrations* from experiment to experiment, as illustrated by the data of Table V. As will appear later, these variations are probably to be attributed to variations in the operation of the water secretion mechanism.

TABLE V

	Experiment 6	Experiment 8	Experiment 9
Rate of secretin administration per 5 min. per kg. weight reduced:	From 0.8 to 0.2 mg. (i.e., by a factor of 4)	From 0.47 to 0.12 mg. (i.e., by a factor of 4)	From 0.7 to 0.23 mg. (i.e., by a factor of 3)
Decrease in rate of H <sub>2</sub> O secretion per 30 min. per kg. weight:	From 1.4 to 0.5 cc. (i.e., by a factor of 3)	From 0.7 to 0.09 cc. (i.e., by a factor of 8)	From 0.9 to 0.12 cc. (i.e., by a factor of 7)
Change in protein nitrogen concentration of pancreatic juice:	Increased from 84 to 75 mg. per cent	Increased from 159 to 346 mg. per cent	Increased from 72 to 175 mg. per cent

As in experiments with a constant rate of secretin administration, the protein nitrogen concentration was considerably diminished toward the end of long experiments, while the concentrations of the metals, the ash, and the non-protein nitrogen remained essentially constant.

The data for the last two samples of Table II indicate that the composition of the secretion obtained in response to secretin administration is not appreciably changed on administration of a relatively large dose of atropine (4 mg., and 45 min. later 1 mg., to a dog of 7.5 kg.). Conclusive evidence was obtained by carrying out an experiment in which atropine was administered throughout; the composition of the secretion obtained, and its changes on varying the rate of administration of secretin, were similar to those of experiments in which atropine was not given.

(c) *The effect of a period of rest (2 hr.) on the composition of pancreatic juice obtained under a constant rate of secretin administration (Table III).* The data show that the concentration of protein nitrogen in the secretion is considerably increased by the 2-hr. period of rest. As will appear in the following paper, this behaviour is probably to be accounted for by a relatively rapid synthesis of protein material within the glandular cells. The increase in the

non-protein nitrogen concentration after the rest period is not necessarily to be explained on the basis of the prolonged interruption of secretory activity, as such an increase is sometimes observed toward the end of experiments with continuous secretory activity. The concentration of chlorides was somewhat higher after the rest period, and the acid combining power somewhat lower, while the rate of secretion of water was not greatly changed. The fact that the acid combining power is not increased after the rest period, while the protein nitrogen concentration is markedly so, has already been referred to in (a).

### The Relation between Protein Nitrogen Data and the Absorption Spectrum Measurements

In solutions containing a single type of absorbing protein, the  $\epsilon c$  values obtained from absorption spectrum measurements will be proportional to the protein nitrogen concentrations, providing Beer's law holds. This is the situation, essentially, in the submaxillary saliva of the cat obtained by chorda tympani stimulation (11). If several different types of absorbing protein are present in comparable amounts, however, and if Beer's law holds, the ratio of the observed  $\epsilon c$  value to the protein nitrogen concentration ( $PN$ ) will be given by

$$\epsilon c/PN = (\epsilon_1 c_1 + \epsilon_2 c_2 + \epsilon_3 c_3 + \dots) / (p_1 c_1 + p_2 c_2 + p_3 c_3 + \dots)$$

where  $\epsilon_n$ ,  $c_n$  and  $p_n$  represent respectively the extinction coefficient, the concentration, and the nitrogen fraction of the  $n$ th protein. In this equation,  $\epsilon c/PN$  becomes constant if  $c_1 = \alpha c_2 = \beta c_3$  etc., where  $\alpha$ ,  $\beta$ , . . . are constants. If this is not so, constancy of  $\epsilon c/PN$  can be attained only if  $\epsilon_1/p_1 = \epsilon_2/p_2 = \epsilon_3/p_3 = \dots = \text{a constant}$ ; such a relation between the  $\epsilon$ 's and  $p$ 's is rather unlikely to occur in complex solutions. Hence if  $\epsilon c/PN$  is observed to be constant for solutions containing several types of absorbing protein, the most probable explanation is that the different proteins are always present in constant proportions, no matter what the concentration of total protein may be. Some observed values for the  $\epsilon c/PN$  ratio of pancreatic secretion obtained in various experiments (*cf.*, Tables I, II and III) are given in Table VI.

The data show that  $\epsilon c/PN$  for samples of pancreatic juice is a constant, even though the total protein content varies over a wide range. In view of the statements made above, this finding indicates that either (a) only one type of absorbing protein is present, or (b) if several types are present, they are always in constant proportions.

It is known that the three principal pancreatic enzymes, namely, trypsin, lipase, and amylase, are secreted in constant proportions (1, 2, 3, 8, 16, 20). If only one type of absorbing protein is present, then presumably it must act as a "carrier" for the different enzymatically active groups. If, however, the enzymes are to be associated with different proteins, the observed constancy of the  $\epsilon c/PN$  ratio supports the finding of these investigators. Further-

more, it indicates that not only the different "active" types of protein, but also any inert types present, are always in constant proportions in the secretion. This agrees with the observation that the relative proteolytic power of different samples of pancreatic juice corresponds closely with the relative nitrogen concentrations (5).

TABLE VI  
Observed  $\epsilon$ /PN ratios

Exp. No.	<i>PN</i> (mg. %)	<i>ε</i>	<i>ε/PN</i>	Mean	Exp. No.	<i>PN</i> (mg. %)	<i>ε</i>	<i>ε/PN</i>	Mean
4	212	23.5	0.111	0.116	6	118.0	14.1	0.119	0.122
	183	18.8	0.103			84.3	10.2	0.121	
	172	20.5	0.120			74.8	8.9	0.119	
	137	17.1	0.124			96.9	11.4	0.118	
	146	17.7	0.121			77.3	9.2	0.119	
	127	14.4	0.114			96.9	11.4	0.118	
	153	18.0	0.118			74.5	9.2	0.123	
5	341	36.0	0.106	0.110	7	55.4	6.7	0.121	0.115
	227	24.5	0.108			62.2	8.4	0.134	
	185	18.7	0.102			58.8	7.5	0.129	
	160	15.8	0.098			174.0	22.4	0.128	
	134	15.6	0.116			150.0	17.0	0.114	
	118	13.1	0.110			355.0	(35.0)	(0.099)	
	110	13.1	0.120			172.0	19.4	0.113	
	108	13.1	0.122			225.0	27.1	0.121	

### Discussion

The data of the preceding section are believed to describe the operation of a glandular secretion mechanism controlled by secretin alone, since the action of the vagi was eliminated by cutting them or by atropine administration, and the passage of gastric juice and bile into the duodenum was prevented.

The concentrations of sodium, potassium, and calcium in the pancreatic juice secreted under secretin administration were found to be independent of the degree of activity of the gland, as were also the sum of the concentrations of chloride and bicarbonate, and the non-protein nitrogen concentration. This fact suggests that the pancreatic membranes offer little resistance to the passage of simple inorganic ions, since, if it were not so, some dependence might be expected purely on analogy with the behaviour of the submaxillary gland (11). This view is supported by the fact that the concentrations of sodium and potassium in the pancreatic juice are nearly the same as in the blood serum (6, 7). The low concentration of calcium in the secretion as compared with that in the serum is undoubtedly due to the fact that a large part of the calcium in the latter fluid is normally bound in some way to protein molecules and so is in "non-diffusible" form (see (18)). With respect to the results of experiments in which salts are injected into the blood stream (*cf.*, 6, 7), it may be noted that the tendency of some substances at least to

form non-diffusible complexes makes it difficult to interpret such data in terms of membrane permeability for simple inorganic ions.

The acid combining power values given in the tables express very nearly the bicarbonate concentrations. The increase which is observed on increasing the glandular activity (*i.e.*, the secretin injections) is interpreted as due to the increased rate at which carbon dioxide is formed as a metabolic product within the gland. On this basis, a part at least of the bicarbonate has its immediate origin within the secretory cells.

The observed increase in the bicarbonate concentration of the secretion was accompanied by a decrease in the chloride concentration, such that the sum of the two in milliequivalents per litre remained essentially constant. It is significant that in order to obtain a constant sum the concentrations must be expressed in this unit (or in millimoles per litre). It indicates that the number of chloride and bicarbonate ions, or the number of anions per unit volume (other anions are present in concentrations that are small compared to chloride plus bicarbonate), is maintained practically constant. The following interpretation is suggested. A difference in net ionic charge on two sides of a membrane results in an electrical potential difference across it; this potential difference facilitates the passage into the cell of ions of one sign, but hinders that of ions of the other. An increased rate of formation of anions (bicarbonate) within a cell due to increased metabolism results in a change in the potential difference favouring more rapid passage of cations (sodium, potassium, calcium) but retarding the passage of anions (chloride, bicarbonate) from the blood stream or tissue fluids. Since the cations already pass easily through the membrane at the maximum rate (the concentrations in the secretion are comparable with those in the serum), the principal effect is a retarding of the passage of anions, and so appears as a decrease in the chloride concentration of the secretion.

A full discussion of the data on the secretion of protein material is reserved for the following paper. It may be noted here, however, that in some experiments (*e.g.*, No. 6) a decrease in the rate of administration of secretin was accompanied by a *decrease* in the protein nitrogen concentration, while in others (*e.g.*, Nos. 8 and 9) it was accompanied by a considerable *increase* in the protein nitrogen concentration. This difference in behaviour is attributed to a difference in the operation of the water secretion mechanism, since, as will be shown in the following paper, the *output* of protein material exhibits a uniform behaviour.

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## DETERMINATION OF NITRITE, NITRATE, AND CHLORIDE IN CURED MEAT AND CURING PICKLE<sup>1</sup>

By W. H. WHITE<sup>2</sup>

### Abstract

An extract suitable for the quantitative determination of nitrite, nitrate, and chloride in cured meat was prepared by freezing and thawing the sample, followed by extraction with hot water. The sulphanilic acid- $\alpha$ -naphthylamine hydrochloride method, applied to the photoelectric colorimeter, was suitable for the determination of nitrite in meat extract and curing pickle. A number of factors that affect this reaction were investigated. The nitrate content of cured meat and curing pickle was determined by the phenoldisulphonic acid method, slightly modified and applied to the photoelectric colorimeter. The chloride content of meat extract was determined, either by direct titration with potassium chromate as indicator, or by Volhard's procedure, after the removal of protein by ignition or wet oxidation. The latter method should be used if accuracy within 5% is desired. Direct titration was satisfactory for the determination of chloride in curing pickle.

The precision of the above methods is illustrated by the following average deviations of individual determinations from their means, as computed from 25 or more duplicate determinations, and expressed as a percentage of the amount present, for meat and pickle respectively: chloride,  $\pm 0.20\%$  and  $\pm 0.02\%$ ; nitrate  $\pm 0.70\%$  and  $\pm 0.30\%$ ; nitrite,  $\pm 1.7\%$  and  $\pm 0.1\%$ .

### Introduction

Preliminary to a survey of Wiltshire curing practice in Canada, an extensive study was made of methods for determining the nitrite, nitrate, and chloride content of cured meat and curing pickle. Many of the available procedures were found to be too laborious, or not adaptable to routine analysis, while others lacked precision or accuracy. A study was therefore undertaken with the object of developing procedures applicable to routine work, and capable of giving satisfactory reproducibility and accuracy.

Methods of the A.O.A.C. (8, pp. 354-357) for the determination of chloride, nitrate, and nitrite in cured meats require separate portions of the sample for each determination. When all three components are to be determined in the same sample, it is desirable that one extract should serve for the complete analysis. Attention was therefore given to suitable methods for preparing such an extract.

Since the concentrations of nitrite and nitrate in a cured meat extract of this kind are rather low, considerable attention was given to colorimetric methods sensitive to small quantities of these constituents. Several of the

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difficulties inherent in such procedures were overcome by the use of a photo-electric colorimeter. This paper describes a new method for extracting salts from cured meat, and advantageous modifications of existing procedures for determining the components of this extract and of curing pickle.

### Preparation and Extraction of the Sample

#### *Cured Meat*

A number of possible methods for suitably preparing the sample for aqueous extraction were investigated. There is evidence from related studies that extraction is facilitated by grinding the sample with sand (11) or by freezing and thawing it (2). The effect of these two treatments was studied by passing a sample of Wiltshire bacon through a food chopper adjusted for fine grinding, until it was thoroughly ground and mixed. This material was then divided into four sub-samples, and treated as follows: (i) untreated; (ii) minced and ground with a coarse silica sand; (iii) minced, frozen, and thawed; and (iv) minced, frozen, thawed, and ground with sand. Each sub-sample was again divided for extraction with hot (boiling) or cold water. After adding 100 ml. of water to 15 gm. of each sample, the whole was shaken for 2 hr., centrifuged and the extract decanted. Nitrite and chloride determinations on each extract gave a measure of the completeness of the extraction. The average values for complete duplicate determinations are given in Table I.

TABLE I

EFFECT OF PRELIMINARY TREATMENT OF THE SAMPLE AND TEMPERATURE OF THE WATER ON THE EXTRACTION

Procedure	NaNO <sub>2</sub> , %		NaCl, %	
	Cold extraction	Hot extraction	Cold extraction	Hot extraction
Minced	0.0022	0.0023	3.36	3.51
Minced and ground with sand	0.0021	0.0025	3.43	3.63
Minced, frozen, and thawed	0.0023	0.0027	3.69	3.70
Minced, frozen, thawed, and ground with sand	0.0025	0.0026	3.58	3.69

The results show that mincing, freezing, and thawing the sample give the best extraction of the salts. Although grinding with sand is better than mincing alone, no definite advantage is gained by its use in the freezing and thawing procedure. More nearly complete extraction was obtained with hot than with cold water.

The extraction proper may be accomplished by two more or less distinct methods. The first may be termed "complete" extraction, and involves extraction by shaking with successive portions of the hot solvent until the process is complete. The decanted solutions are combined, made up to volume, and an aliquot analyzed. The second method may be termed

"equilibrium" extraction, since the sample is placed in a volumetric flask with hot water, shaken for a given period, made up to volume, and a suitable aliquot taken for analysis. Such a method assumes that an equilibrium will be attained in which the quantity of salts contained in unit volume occupied by the meat will be the same as that in unit volume of aqueous extract. In this method correction for volume occupied by the dry matter may be made, but is usually negligible.

A comparison of the two procedures showed that five extractions were required for the complete removal of the salts, and that equilibrium was reached after shaking for 2 hr. Although "complete" extraction appears to have a sounder basis, the "equilibrium" method involves fewer manipulations, and is consequently more suitable for routine analysis.

A number of miscellaneous experiments on other types of extraction, and on factors that might affect extraction, were conducted. Refluxing meat samples with water gave lower results than the above procedure, although the liquid was kept definitely alkaline in order to prevent the loss of nitrite (4). Adjusting the pH of minced, frozen, and thawed samples with lactic acid or ammonium hydroxide had no beneficial effect on the "equilibrium" method of extraction.

The following procedure was therefore adopted for the extraction of salts from cured meat:

Lean meat from the sample to be analyzed was thoroughly minced, and mixed by several passages through a food chopper adjusted for fine grinding. After freezing and thawing, a 10-gm. sample was weighed into a 100-ml. beaker, a small quantity of cold water added, and the mixture worked into a paste. This was transferred to a 200-ml. wide-neck volumetric flask, and a sufficient quantity of boiling water added to bring the final volume to approximately 150 ml. The flask was stoppered, and shaken vigorously for 2 hr. in a shaking machine equipped with a steam chest to maintain the flask at a temperature of 80° C. or higher. The contents of the flask were then brought to room temperature within a period of one-half to one hour, made up to volume, shaken thoroughly, and filtered through a large fluted filter. Portions of this one extract, after suitable dilutions, served for nitrite, nitrate, and chloride determinations.

For the most part, clear extracts are obtained which do not require the use of any protein precipitant as a clarifying agent. The results of a large number of determinations have shown the method to be quite satisfactory, both in its applicability to routine work, and in its precision and accuracy. A comparison of the results given by this method with those obtained by the standard procedures of the A.O.A.C. (8, pp. 354-357) will be given later.

### *Curing Pickle*

Since curing pickle already contains the salts to be determined in the form of a solution, no preliminary treatment of the sample is necessary.

## Application of the Photoelectric Colorimeter to the Determination of Nitrite and Nitrate

Although the procedures to be described later are suitable in most cases for visual colorimetry, they have been adapted especially to the photoelectric colorimeter. The comparison of colour intensities by such an instrument eliminates many of the objections levelled at colorimetric methods, such as the necessity of preparing a number of standards, their possible variation from day to day, and the inadequacy of the human eye for accurate comparison of varying intensities of the same colour. The photoelectric colorimeter used in these investigations was that designed by Evelyn (3). If the reaction obeys the Lambert-Beer law, a constant relating the transmitted light and the concentration of the constituent may be calculated. If it does not obey this law, a calibration chart must be prepared by plotting values for concentration against corresponding galvanometer deflections. Details necessary for the use of the photoelectric colorimeter in these determinations are given below.

Spectrophotometric examination of the red colour developed in the sulphanilic acid- $\alpha$ -naphthylamine hydrochloride method for the determination of nitrite indicated that a Rubicon No. 520 filter, transmitting 95% of the incident light in the range 4950Å to 5500Å, was suitable. The results of a complete series of triplicate determinations on a number of standard solutions of sodium nitrite varying in concentration from approximately  $1 \times 10^{-3}$  to  $1 \times 10^{-4}$  mg. per ml. of solution gave an average value of 1.0 for  $K_1$ , and showed that the Lambert-Beer law was applicable (Table II).

TABLE II  
VALUES OF CORRECTED GALVANOMETER DEFLECTION AND OF  $K_1$  FOR  
THE DETERMINATION OF NITRITE

Conc. of $\text{NaNO}_2$ , (mg. $\times 10^{-3}$ per ml.)	Corrected galvanometer deflection	$K_1$	Conc. of $\text{NaNO}_2$ , (mg. $\times 10^{-3}$ per ml.)	Corrected galvanometer deflection	$K_1$
1.37	6.00	0.89	0.319	48.00	1.00
0.910	13.25	0.97	0.296	50.50	1.00
0.683	20.75	1.00	0.273	52.75	1.02
0.637	23.25	1.00	0.251	56.50	0.99
0.546	28.75	0.99	0.228	59.25	1.00
0.501	32.50	0.97	0.182	65.00	1.03
0.455	35.00	1.00	0.091	80.00	1.06
0.410	38.50	1.01	0.061	86.25	1.05
0.364	43.50	1.00	0.036	91.50	1.08
0.347	45.45	0.99			

A Rubicon No. 420 filter, transmitting 95% of the incident light in the region 3800Å to 4600Å, was used for the intensity measurements of the yellow colour developed in the phenoldisulphonic acid method for nitrate. Preliminary investigations indicated that the method gave low values compared to other procedures, and did not obey the Lambert-Beer law (possibly

due to occlusion of nitrate by the bulky precipitate formed). In order to overcome these difficulties somewhat, a calibration curve was prepared with standard sodium nitrate solutions, to which sodium chloride was added in order to simulate, in part, the conditions existing in meat extract and curing pickle. The results are illustrated graphically in Fig. 1 for the range from 0 to 1.00 mg. of sodium nitrate, and for a colour dilution of 100 ml.

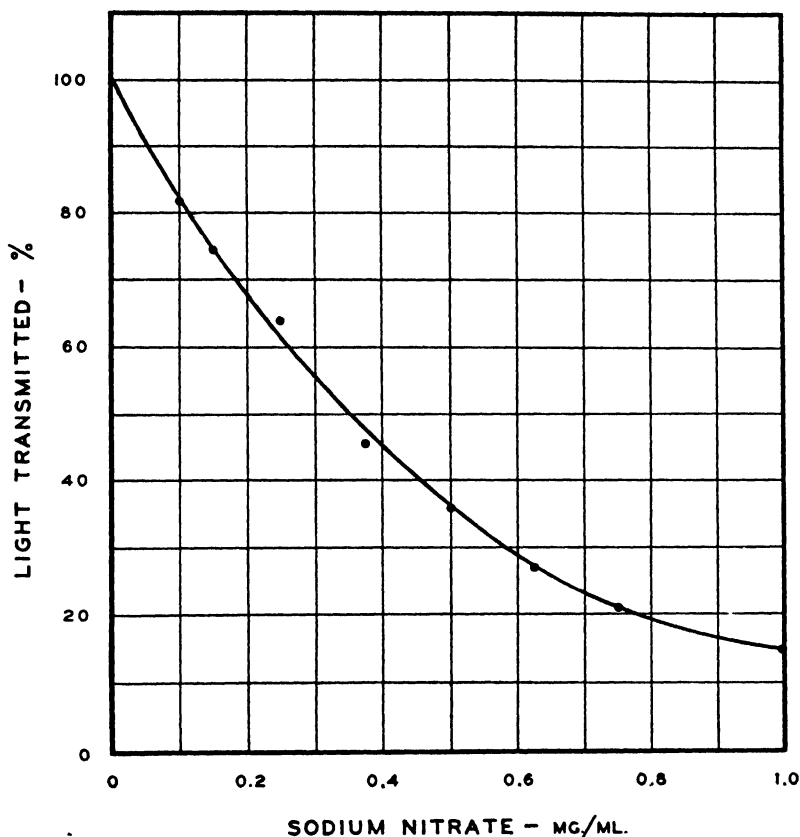


FIG. 1. Calibration curve for the determination of nitrate by the phenoldisulphonic acid method.

#### DETERMINATION OF NITRITE

##### *Cured Meat*

Of a number of colorimetric methods available for the determination of nitrite, that proposed by the A.O.A.C. (8, p. 506) was selected and found to be entirely satisfactory. To 10 ml. of meat extract, diluted to contain approximately  $1.0 \times 10^{-3}$  to  $1.0 \times 10^{-4}$  mg. of sodium nitrite per ml. of solution, was added one drop of concentrated hydrochloric acid, one ml. of a solution of sulphanilic acid, and one ml. of  $\alpha$ -naphthylamine hydrochloride, and the tube shaken. The blank solution was prepared in the same way, but the sulphanilic acid was omitted.

The effect of a number of factors on rate of development and maximum intensity of the colour was investigated. The rate was studied by making readings of the galvanometer deflection against time for a number of solutions varying in concentration of sodium nitrite from  $0.036 \times 10^{-3}$  mg. to  $1.37 \times 10^{-3}$  mg. per ml. of solution. The results for each of the 19 concentrations indicated that the rate was very rapid during the first 5 min., and that readings made 15 min. after the addition of the reagents corresponded to maximum colour intensity. It was observed that, for the more dilute solutions, the colour had faded only very slightly even after a period of 24 hr. However, for the more concentrated solutions, the intensity had decreased considerably at the end of this period. A few of the typical curves obtained are shown in Fig. 2.

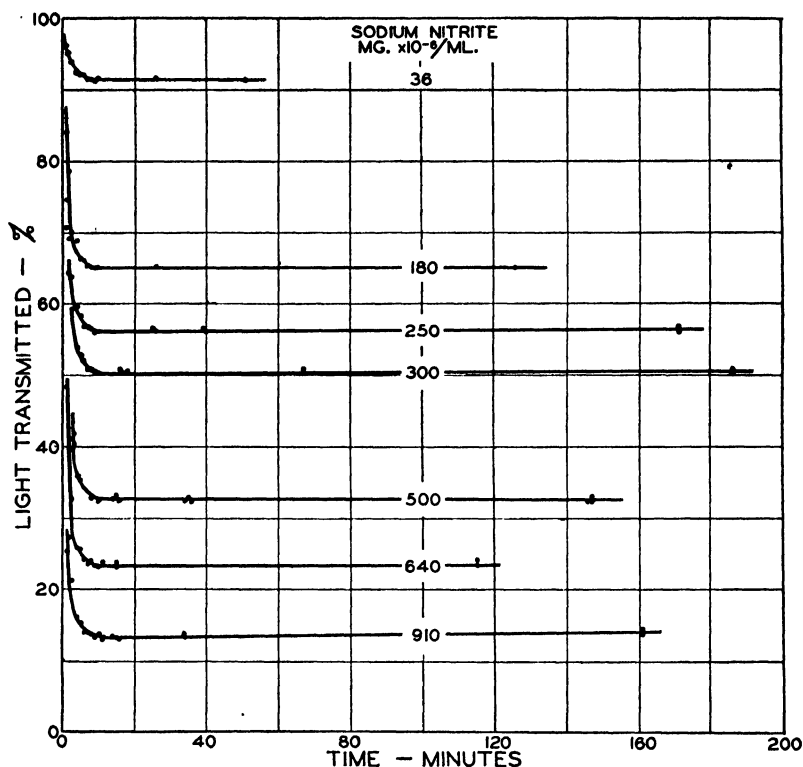


FIG. 2. Colour development at various concentrations of nitrite in the sulphanilic acid- $\alpha$ -naphthylamine hydrochloride method.

Other factors investigated were temperature, light, sodium chloride, pH, concentration of reagents, their age, and the method of their addition. The results are shown in Table III. The colour of a solution held in a steam bath developed more rapidly than that of one kept at room temperature. After reaching its maximum in 4 min., the intensity subsequently decreased, indicating that the chromogen was being either precipitated or destroyed.

Both the rate and maximum intensity of colour were increased by decreasing the intensity of incident light during colour development.

TABLE III

EFFECT OF A NUMBER OF FACTORS ON THE RATE AND FINAL INTENSITY OF THE COLOUR DEVELOPED IN THE SULPHANILIC ACID- $\alpha$ -NAPHTHYLAMINE HYDROCHLORIDE METHOD FOR NITRITE

Factor studied	Experimental details	Conc. of $\text{NaNO}_2$ , (mg. $\times 10^{-3}$ per ml.)	
		Theoretical	Found
Temperature	Room temperature	0.185	0.185
	Heated on steam bath for 5 min.	0.185	0.181
	Heated on steam bath for 268 min.	0.185	0.095
Light	Sunlight	0.185	0.170
	Laboratory conditions	0.185	0.185
	Darkness	0.185	0.189
Sodium chloride	10% solution	0.185	0.186
	20% solution	0.185	0.187
	30% solution	0.185	0.187
pH	0.95	0.185	0.124
	1.82	0.185	0.185
	6.58	0.185	0.174
Addition of reagents	1 cc. of each, added separately	0.185	0.185
	1 cc., mixed	0.185	0.163
	2 cc., mixed	0.185	0.166
Concentration of reagents	1 cc. of each, added separately	0.185	0.185
	5 cc. of each, added separately	0.185	0.208
Age of reagents	Freshly prepared	0.185	0.185
	One week old	0.185	0.189

The presence of sodium chloride slightly increased the rate of colour development but had no effect, within experimental error, on the intensity of the fully developed colour. There is some indication that the observed increase in rate varies directly with the concentration of sodium chloride. The effect of pH was studied on three solutions adjusted to pH values of 0.95, 1.82, and 6.58, the second being the value used in all previous determinations. The rate of colour development was approximately the same for the two solutions of low pH, but was considerably retarded at the higher value. The final colour intensity was greatest for a pH of 1.82, less for 0.95, and least for 6.58.

Both the rate and final intensity of the colour are decreased by mixing the reagents prior to their addition. On a comparable volume basis, the results indicate that these two properties vary directly with the concentration of reagents employed. A solution of  $\alpha$ -naphthylamine a week old, although considerably discoloured, gave results strictly comparable with those obtained with a freshly prepared solution.

The procedure proposed here for extraction and determination of nitrite gave results approximately 1% lower than that of the A.O.A.C. (8, pp. 356-357), but of equally satisfactory precision. The results of 25 duplicate determinations on smoked and unsmoked Wiltshire bacon, with nitrite concentrations ranging from 0.00023% to 0.013%, showed an average variation about their means of  $\pm 1.7\%$ .

### *Curing Pickle*

The method described above is applicable without further modification to the determination of the nitrite content of "pump", "cover", or "spent" pickle\*. The use of the photoelectric colorimeter makes it unnecessary to remove any protein present which may give colour to the pickle. The results of analyses on 25 pickles of each type showed an average variation about the mean of  $\pm 0.1\%$ ,  $\pm 0.2\%$ , and  $\pm 0.1\%$  for pump, cover, and spent pickle respectively.

### *Cured Meat*

#### DETERMINATION OF NITRATE

The nitrate content of cured meat may be determined either gasometrically or colorimetrically. Since the gasometric method requires a relatively large quantity of nitrate for accurate determination, its use would necessitate the preparation of a more concentrated extract than required for the determination of nitrite and chloride. As this was not desirable, attention was given to colorimetric procedures. Of the several possible methods, only three were thought to merit consideration. Both the brucine (10) and phenoldisulphonic acid methods (8) have been applied to cured meat. A more recently described method (7), depending on the reduction of nitrate to nitrite, and its subsequent determination, was thought to be applicable. Preliminary studies, however, indicated that the brucine and nitrate-nitrite reduction methods were both unsatisfactory because of the inconsistency of the results obtained.

Difficulty was also encountered in obtaining satisfactory results with the phenoldisulphonic acid method described by the A.O.A.C. (8, p. 356). A study of possible causes for the discrepancies observed confirmed a previous finding (1) that the solution should be alkaline during evaporation. The addition of sodium hydroxide serves a further purpose in that any excess silver sulphate present is precipitated. Nitrite interferes and is removed by oxidation to nitrate with potassium permanganate. A suitable method for the preparation of the phenoldisulphonic acid reagent is described in (9, p. 633). The details of the suggested modification of this method as applied to cured meats are given below.

To a 25-ml. portion of the extract in a 100-ml. volumetric flask, one drop of sulphuric acid (1 : 10) is added, followed by 0.6% potassium permanganate solution, drop by drop, until a pink colour remains in the extract for approximately 2 min. Chloride is precipitated with a saturated solution of silver

\* The pickle injected into the sides is designated here as pump, the freshly prepared tank pickle as cover, and that removed from the tank after cure as spent.

sulphate added in slight excess, as indicated by precipitation on the addition of a few drops of *N* sodium hydroxide (carbonate-free). Protein material is then precipitated by adding 2 ml. of saturated basic lead acetate solution, followed by a sufficient quantity of the sodium hydroxide solution to make the solution alkaline to litmus. The flask is shaken thoroughly after the addition of each of the above reagents, the solution made up to volume, shaken, and filtered through a coarse paper until clear. (The use of a fine filter paper will result in sufficient retention of nitrate to affect the results appreciably (5).) A suitable portion of the extract, containing 0.15 to 1.0 mg. of sodium nitrate is pipetted into an evaporating dish, and taken to dryness on the steam bath.

The residue is dissolved in 2 ml. of the phenoldisulphonic acid and, after standing for 10 min., diluted with 25 ml. of cold water. The solution is made definitely alkaline with concentrated ammonium hydroxide (about 10 ml.), and transferred to a Nessler tube graduated at 50 and 100 ml. The contents are made up to either volume, depending on the intensity of the colour, shaken, filtered through a fine paper, and a portion of the filtrate transferred to a comparison tube of the photoelectric colorimeter. The blank solution, used in the initial setting of the colorimeter, is prepared by adding the same quantities of ammonium hydroxide and water to 2 ml. of the phenoldisulphonic acid reagent. As the method determines both the nitrate and nitrite present, the percentage of nitrate is obtained by subtracting that for nitrite from the total. However, the nitrite content of Wiltshire bacon is usually quite low, and may be neglected if nitrate alone is being determined.

This method for the extraction and determination of nitrates gives quite satisfactory reproducibility. The results of 25 duplicate determinations picked at random from a large number of analyses in duplicate of Wiltshire bacon (both smoked and unsmoked) showed an average variation of  $\pm 0.73\%$  about the mean.

#### *Curing Pickle*

The above described method is applicable to the determination of the nitrate content of a suitably diluted portion of curing pickle. In this instance a 0.2% solution of potassium permanganate is used for the oxidation of nitrite to nitrate. The analyses in duplicate of 25 pump, cover, and spent pickles showed an average variation about the mean of  $\pm 0.3\%$ ,  $\pm 0.2\%$  and  $\pm 0.4\%$  respectively.

This colorimetric method was compared with the Schlösing-Wagner gasometric procedure (8, pp. 355-356). The nitrate content of a pump, cover, and spent pickle was determined by each of the two methods. The results shown in Table IV indicate that the phenoldisulphonic acid method gives comparable results for pump, but lower values for spent and cover pickles. This is believed due to the occlusion of nitrate by the rather bulky precipitate formed by protein usually present in the last two types, but either absent, or present in a negligible quantity, in the first. The colorimetric

procedure gives closely reproducible results, but is somewhat lacking in accuracy.

TABLE IV  
COMPARISON OF THE COLORIMETRIC AND GASOMETRIC  
METHODS FOR THE DETERMINATION OF NITRATE

Type of pickle	Analytical procedure	
	Colorimetric	Gasometric
Pump	2.73	2.73
Cover	1.82	1.93
Spent	1.08	1.15

### *Cured Meat*

### DETERMINATION OF CHLORIDE

The chloride content of a suitable portion of the extract may be conveniently determined with high reproducibility and 4 to 5% accuracy by direct titration with 0.1 *N* silver nitrate, using 1 ml. of a 5% solution of potassium chromate (8, p. 507) as indicator.

The results of 25 duplicate determinations, picked at random from a large number made on Wiltshire bacon containing from 1.8 to 6.0% sodium chloride, showed an average variation of  $\pm 0.15\%$  from their means.

A measure of the accuracy of the extraction procedure, and the direct titration method for determination of chloride in the extract were obtained by comparison with the standard procedure of the A.O.A.C. (8, p. 254). Five different samples of Wiltshire bacon were each analyzed in duplicate by the following three methods:

- I. The A.O.A.C. standard method: ignition of the sample followed by Volhard's determination (proteins may be eliminated by wet oxidation also (6)).
- II. Twenty-five ml. of the extract treated as in I.
- III. Direct titration.

The results appear in Table V, together with certain statistical quantities calculated to determine the significance of the observed differences. Although the standard error of duplicates suggests that the direct titration method is the most precise, the observed differences in these errors by the different methods are not statistically significant. However, the differences between the means by the three methods are significantly greater than their standard errors. Comparison of the results obtained with methods I and II show that the extraction phase of II is satisfactory. The higher values obtained with II may be the result of an error introduced by the impenetrable portion of the meat and its water of hydration when the extract is made up to volume. No correction has been made for this in the results presented here. On the

average the direct titration procedure gives results 4.4% too high, probably because of the presence of protein in the extract.

TABLE V

COMPARISON OF THE EXTRACTION PROCEDURE WITH THE A.O.A.C. STANDARD METHOD FOR THE DETERMINATION OF THE CHLORIDE CONTENT OF CURED MEAT

Procedure	NaCl, %		
	I	II	III
Experimental details	Direct ignition of meat; Volhard titration	Extract of meat evaporated and ignited; Volhard titration	Extract of meat directly titrated by Mohr's procedure
1	6.165	6.330	6.565
2	4.540	4.645	4.855
3	3.355	3.490	3.705
4	3.960	4.065	4.250
5	4.135	4.265	4.415
Mean	4.431	4.559	4.758
Standard error of duplicates	0.0140	0.0125	0.0100

### *Curing Pickle*

The chloride content of any of the three types of pickle can be determined with good reproducibility and accuracy by means of the previously described direct titration procedure on a suitably diluted portion of the sample. As an independent investigation had indicated that the amount of protein commonly present in curing pickle has little effect on the Volhard method for chloride, three pickles were analyzed by both this and the direct titration procedure in order to determine the accuracy obtainable with the latter. The results, shown in Table VI, indicate close agreement between the two methods. The reproducibility is also satisfactory, since the values for 75 duplicate analyses had an average variation of  $\pm 0.02\%$  about the mean.

TABLE VI

COMPARISON OF DIRECT TITRATION AND VOLHARD PROCEDURES FOR THE DETERMINATION OF SODIUM CHLORIDE IN CURING PICKLE

Pickle sample	NaCl, %		Deviation of direct titration from Volhard, %
	Direct titration	Volhard	
1	29.1	28.9	+0.69
2	31.2	31.0	+0.65
3	30.9	31.0	+0.32

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# THE SYNTHESIS AND SECRETION OF PROTEIN MATERIAL BY THE PANCREAS<sup>1</sup>

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## Abstract

A mathematical treatment of the process of synthesis of protein material in the pancreas, and its secretion in response to secretin administration, has been developed. It was based on assumptions inferred from experimental data and from analogy with a suggested mechanism for the submaxillary gland, published previously. It leads to expressions that quantitatively describe the protein output in samples of pancreatic juice obtained under widely varied experimental conditions. Inferences concerning the fundamental character of certain glandular mechanisms may be drawn from the treatment. In addition it permits provisional calculation of various factors not directly observable in critical experiments, and suggests further problems in connection with the secretory processes.

## Introduction

This article is mainly concerned with an attempt to interpret the data for the secretion of protein material in the experiments reported in a previous paper (4). As precautions were taken to eliminate effects due to the action of the vagus nerves or of gastric juice or bile on the small intestine in these experiments, it is considered that the data and the following interpretations refer to the behaviour of the glandular mechanism controlled by secretin.

The interpretation involves a mathematical treatment of the processes of synthesis and secretion of protein material. The premises for this treatment were suggested partly by the experimental data, partly by analogy with the behaviour of the submaxillary gland (5) and partly by general considerations. They include the following suppositions. (i) The mechanism responsible for the secretion of protein material and that controlling the secretion of water are largely independent, except as far as they both depend on the presence of secretin for their initiation. As will appear later, this view receives support from the fact that it is possible to describe the protein output in a series of samples by a theory which does not consider the secretion of water. (ii) The synthesis of protein material in the pancreas may take place at a rate comparable to that at which it is secreted in critical experiments. Synthesis is very slow in the submaxillary gland of the dog (1, 2, 3, 6); essentially complete restoration of the granules in a gland which has undergone prolonged stimulation requires from three to six days. Therefore it was permissible to neglect it in a theoretical treatment of the results obtained in critical experiments on that gland (5). When the rate of synthesis becomes comparable to the rate of secretion, however, it is an important factor in determining the

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protein output and must be taken into account. The results of experiments on the pancreas performed with a constant rate of secretin administration (4, Table I) show that the protein output per 30-min. sample decreases with time, but tends to approach a nearly constant value toward the end of the experiment. This tendency toward a constant rate of output is to be expected if synthesis is an appreciable factor, since under constant intensity of the stimulus the processes tend to reach an equilibrium state in which the rates of synthesis and secretion are equal. For reasons that will appear later, equilibrium is probably never fully attained. The strongest evidence in favour of this view of synthesis in the pancreas is that it leads to a quantitative description of the observed data in critical experiments. Synthesis of granule material must naturally occur through a chemical reaction. (iii) The secretion of protein material involves a chemical reaction or chain of reactions within the secretory cells. Attempts to explain the data on other grounds (*e.g.*, as a "washing out" of granule material) were unsuccessful. On this view, the water flow seems to remove the reaction products from the gland, and the process is similar in nature to that deduced for the submaxillary gland (5).

The mathematical treatment leads to equations that furnish a quantitative description of all the observed characteristic features of the protein output in the samples of the widely varied experiments described in the preceding paper. Inferences drawn from it allow some insight into the fundamental nature of the glandular mechanisms. In addition, it permits the calculation of various factors not directly observable in critical experiments, and is rich in suggestions for further investigations.

### Formulation of the Theory

The theoretical treatment consists in obtaining velocity equations to describe the secretion and synthesis reactions, as follows:—

*The secretion reaction.* It is assumed that, on the influx of secretin into the cells, a chain of reactions is set up. If secretin and some other primary substance or substances take part in the first step, and if the last step results in a transformation of granule material to a form or forms readily carried out of the cells by the flow of water, then a simple form of the velocity equation describing the transformation of granule material is

$$\frac{dN}{dt} = -k^1(A_1A_2 \dots)(B_1B_2 \dots)N\sigma, \quad (1a)$$

where  $\sigma$  and  $N$  denote respectively the amounts of secretin and granule material present in the gland at the time, and  $A_1, A_2 \dots, B_1, B_2 \dots$  represent the amounts of other primary substances involved in the various steps of the reaction;  $k^1$  is a constant. The equation assumes that the reaction is irreversible, and that the essential products of each step in the chain react practically as fast as they are formed. Over periods of time for which the amounts of the substances  $A_1, A_2 \dots, B_1, B_2 \dots$ , remain essentially

constant, the velocity equation reduces to

$$\frac{dN}{dt} = -kN\sigma, \quad (1)$$

where  $k$  is a constant. The term granule material, as used in this paper, refers simply to the parent substance of the secreted protein material.

*The synthesis reaction.* In order that the amount of granule material in the gland should not increase indefinitely it is assumed that the synthesis reaction is reversible. If a number of substances present in amounts  $D$ ,  $E$ ,  $F$  . . . . combine to form granule material, present in amount  $N$ , a simple form of the velocity equation is

$$\frac{dN}{dt} = \beta \{ (DEF \dots / D_0 E_0 F_0) N_0 - N \}, \quad (2a)$$

where  $\beta$  is a constant, and  $N_0$ ,  $D_0$ ,  $E_0$ ,  $F_0$ , . . . . denote the amounts of the corresponding substances present at the equilibrium point of the reaction. It may be pointed out that as the granule material may not be present in solution, possibly only the surface layers take part in the reaction; on this basis  $N_0$  is defined as the amount of granule material capable of reacting at the equilibrium point of the reaction. A similar qualification holds in the definition of  $N$ . If the synthesis reaction in the gland is practically in the equilibrium state after a long period of rest, then for a period of subsequent stimulation such that the amounts of the substances  $D$ ,  $E$ ,  $F$ , . . . are essentially constant, the velocity equation (2a) reduces to

$$\frac{dN}{dt} = \beta(N_0 - N). \quad (2)$$

The above equations lead to expressions for the protein output in experimental samples without any further assumptions. As will be shown later, these expressions are in remarkably good agreement with the widely varied data of the preceding paper.

The total rate of change of granule material in the gland at any time  $t$  is given by combining Equations (1) and (2), keeping in mind the limitations imposed on these equations by the conditions of simplification from (1a) and (2a). It is

$$\frac{dN}{dt} = \beta N_0 - (\beta + k\sigma)N. \quad (3)$$

On integration, (3) becomes

$$N = N_0 \frac{\beta}{\beta + k\sigma} - \frac{I}{\beta + k\sigma} \cdot e^{-(\beta + k\sigma)t}, \quad (3a)$$

where  $I$  is a constant of integration. Theoretical expressions predicting the behaviour under different sets of experimental conditions are derived from (3a) and other preceding equations, as follows:

(a) *Experiments consisting of an uninterrupted series of samples obtained with a constant rate of administration of secretin (e.g., (4, Table I)).* For these

experiments,  $N = N_0$  when  $t = 0$ , which determines the integration constant  $I$  of (3a). Substituting the value of  $I$  obtained from this condition in (3a), the amount of granule material  $N$  left in a gland that has been secreting under an effectively constant rate of administration of secretin for a time  $t$  from the start of the experiment, is given by

$$N = N_0 \left[ \frac{\beta}{\beta + k\sigma} + \frac{k\sigma}{\beta + k\sigma} \cdot e^{-(\beta + k\sigma)t} \right]. \quad (4)$$

According to Equation (1) the amount of protein material ( $\Delta N$ ) secreted during this time is

$$\Delta N = \int_0^t k\sigma N \cdot dt.$$

Substitution of the value of  $N$  from (4), and integration, gives

$$\Delta N = N_0 \frac{k\sigma}{\beta + k\sigma} \left[ \beta t + \frac{k\sigma}{\beta + k\sigma} \cdot (1 - e^{-(\beta + k\sigma)t}) \right]. \quad (5)$$

The amount  $\delta N$ , secreted in a sample taken between times  $T$  and  $(T + \Delta T)$  of this period, is of course

$$\delta N = \Delta N_{(T+\Delta T)} - \Delta N_T. \quad (6)$$

(b) *Experiments consisting of an uninterrupted series of samples obtained under varied rates of administration of secretin (e.g., (4, Table II)).* For these experiments,  $\beta$  and  $N_0$  are considered to have the same value throughout, but  $k\sigma$  changes on each change in the rate of administration of secretin. In the initial group of samples in which the rate of administration is kept constant, Equations (4) to (6) apply. In any subsequent group of samples obtained under constant stimulus, however, the following equations apply. For such a group the integration constant of (3a) is determined from the condition, when  $t = 0$ ,  $N = N_{i-1}$ , where  $N_{i-1}$  denotes the amount of granule material present in the gland at the start of the period, and time is reckoned from this point. The amount of granule material left in the gland at a time  $t$  after the start of this period is, from (3a),

$$N = N_0 \frac{\beta}{\beta + k\sigma} (1 - e^{-(\beta + k\sigma)t}) + N_{i-1} \cdot e^{-(\beta + k\sigma)t}. \quad (7)$$

The amount of protein material secreted during a time  $t$  from the start of the period is obtained in a way similar to (5) above, as

$$\Delta N = \frac{k\sigma}{\beta + k\sigma} \left[ N_0 \beta t + \left( N_{i-1} - \frac{\beta}{\beta + k\sigma} N_0 \right) (1 - e^{-(\beta + k\sigma)t}) \right]. \quad (8)$$

The amount secreted in a sample taken between times  $T$  and  $(T + \Delta T)$  of the period is given by an equation of the same form as (6). It may be pointed out that  $N_{i-1}$  is not a new variable since it may be expressed in terms of

$N_0$ ,  $\beta$ , and the  $k\sigma$ 's; the expression is too complicated for practical use, however, and it is easier to calculate the amount of protein material left in the gland at the end of the first period from (4), and to use this as  $N_{i-1}$  for the second period. Similarly the value of  $N_{i-1}$  for the third period may be calculated from (7) applied to the second group of samples, and so on.

(c) *Experiments in which the administration of secretin is stopped at some point, and the gland permitted to rest for an appreciable period before resuming stimulation (e.g., (4, Table III)).* The progress of restoration of granule material during a rest period is described by the equation resulting from the integration of (2). The constant of integration is determined from the condition, when  $t = 0$ ,  $N = N_r$ , where  $N_r$  denotes the amount of granule material in the gland at the start of the rest period, and time is reckoned from this point. Accordingly, the amount of granule material  $N_\theta$  present in the gland at a time  $\theta$  after stopping the secretin administration, is

$$N_\theta = N_0 - (N_0 - N_r) \cdot e^{-\beta\theta}. \quad (9)$$

The protein output in the samples obtained on resumption of the administration of secretin is given by Equation (8) with  $N_{i-1}$  replaced by  $N_\theta$ . The value of  $N_r$  is of course calculable from Equation (4) or (7), depending on the conditions of stimulation before the rest period.

*Additional formulae.* While Equations (4) to (9) are those mainly concerned in a comparison of theory and experiment, the following expressions have some interest.

(a) After prolonged secretion under a constant rate of administration of secretin, the amount of protein material secreted during a time  $\Delta T$  approaches a constant value  $\delta N_c$ , which is given by

$$\delta N_c = N_0 \frac{\beta k \sigma}{\beta + k \sigma} \cdot \Delta T. \quad (10)$$

The amount of material synthesized during the same time is given by the same expression. It is to be noted that  $\delta N_c$  depends on the rate of administration of secretin.

(b) The amount of granule material  $\Delta N_s$ , synthesized in a time  $t$  from the start of an experiment in which the rate of administration of secretin is kept constant, is

$$\Delta N_s = N_0 \frac{\beta k \sigma}{\beta + k \sigma} \left[ t - \frac{1}{\beta + k \sigma} \cdot (1 - e^{-(\beta + k \sigma)t}) \right]. \quad (11)$$

The rate of synthesis at any time is given by Equation (2).

(c) The amount of granule material synthesized in a time  $t$  after changing the rate of administration of secretin is

$$\Delta N_s = \frac{\beta}{\beta + k \sigma} \left[ N_0 k \sigma t - \left( N_{i-1} - \frac{\beta}{\beta + k \sigma} N_0 \right) (1 - e^{-(\beta + k \sigma)t}) \right]. \quad (12)$$

*Remarks on the comparison of theory and experiment.* In experiments of type (a) the protein output in the various samples is described in terms of the unknown constants  $N_0$ ,  $\beta$ , and  $k\sigma$ . The values for the constants may be determined from the observed output for three samples of a series, and these values may then be used to calculate the output in all the samples of the series. It may be added that the *relative* outputs in the samples of a series depend on only two unknowns,  $\beta$  and  $k\sigma$ , and so are fixed by the observed output in any two samples of a series.

In experiments of type (b),  $k\sigma$  has a different value for each rate of administration of secretin. If the initial period of constant stimulus be sufficiently long, values for  $N_0$  and  $\beta$ , and for  $k\sigma$  for this period may be determined as for experiments of type (a). The amount of granule material in the gland at the end of the first period may then be calculated from Equation (4) and used as  $N_{i-1}$  in Equation (8) for the second period. One then finds a value of  $k\sigma$  that satisfies the data of the second period. The value of  $N_{i-1}$  for the third period may then be found from Equation (7), and the procedure repeated for the third and successive periods. This procedure—rather than a straightforward assumption of proportionality between  $k\sigma$  and the rate of secretin administration—was adopted for two reasons: firstly, because the simple form of Equation (1) cannot be expected to hold indefinitely as a good approximation of (1a), and secondly, because it does not assume a strict proportionality between the amount of secretin injected and the amount present in the gland. As will be shown later, the  $k\sigma$  values so obtained are closely proportional to the rate of administration of secretin, except toward the end of a long experiment, where a gradual departure occurs in the direction expected if Equation (1) fails to approximate Equation (1a) at this time.

### Some Characteristic Features of the Predicted Behaviour

In order to illustrate more clearly some of the more striking features of the behaviour of a system obeying the preceding equations, the following hypothetical experiment is considered. The calculations were made with constants determined from typical experiments, and so have a general significance. The experimental conditions and predicted behaviour are indicated in Fig. 1.

The following predicted features of the behaviour of the observable factor (*i.e.*, the protein output per sample) are to be especially noted, as they will be referred to in the following section.

- (i) Under constant intensity of the stimulus the protein output per 30-min. sample decreases, and finally approaches a constant value ( $t = 0$  to  $t = 150$ ).
- (ii) With reduced intensity of the stimulus the protein output per 30-min. sample is reduced ( $t = 150$  to  $t = 240$ ).
- (iii) With constant reduced intensity of the stimulus the protein output per 30-min. sample gradually increases and approaches a constant value ( $t = 150$  to  $t = 240$ ).

(iv) On the resumption of the initial intensity of the stimulus the protein output is greater than in the last sample of the first group, but decreases and gradually approaches the same constant value as the first group ( $t = 240$  to  $t = 330$ ).

(v) On the resumption of the initial intensity of the stimulus after an appreciable period of rest, the protein output per sample is considerably increased, but falls rapidly on continued stimulation and tends to reach the same constant value as the first group ( $t = 570$  to  $t = 660$ ).

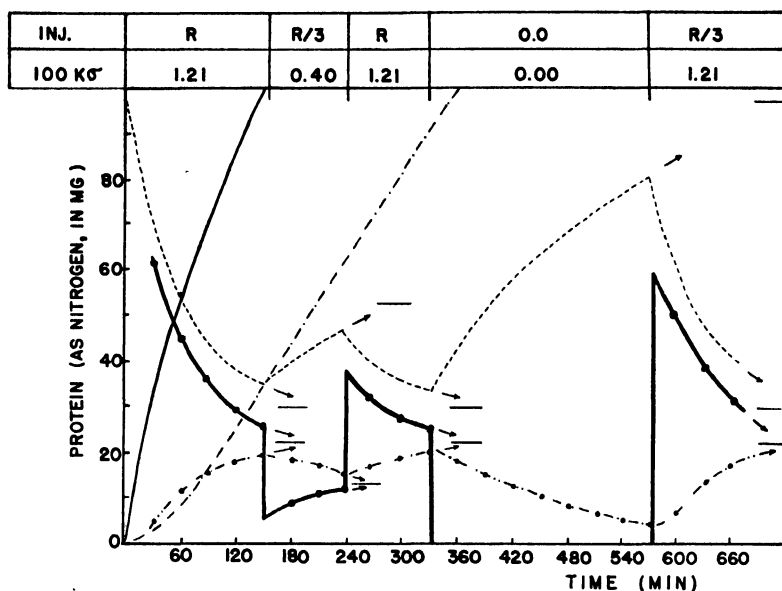


FIG. 1. Some predicted features of a hypothetical experiment as calculated from Equations (5) to (14), with typical values for  $\beta$ ,  $N_0$ , and  $k\sigma$ . As indicated at the top of the figure, five 30-min. samples are obtained with a constant rate ( $R$ ) of injection of secretin, then three samples with a rate  $R/3$ , then three with the initial rate; the secretin administration is stopped after 330 min., and the gland allowed to rest for 4 hr., after which secretin is again injected at the original rate.  $N_0$  and  $\beta$  were taken to be 98 mg., and  $0.51 \times 10^{-2}/\text{min.}$  respectively. The curves have the following significance:

- mg. of protein nitrogen secreted in each 30-min. sample ( $\times 2$ ).
- - ● - - mg. of protein (as nitrogen) synthesized during each 30-min. period ( $\times 2$ ).
- - - - - mg. of granule material (as nitrogen) present in the gland (see remarks concerning the definition of  $N_0$ ).
- total mg. of protein nitrogen secreted from the start of the experiment. Note that this becomes greater than  $N_0$  in about 2½ hr.
- - - - - total mg. of protein synthesized (as nitrogen).

The arrows point to lines representing the equilibrium values which would be attained on the basis of the simplified theory if the stimulus of the immediately preceding period were maintained.

Each of these characteristic features is observed in experiments (next section) and is quantitatively described by the theory. It must be kept in mind, however, that these features are predicted by the theory which uses Equations (1) and (2) as approximations of (1a) and (2a).

Some predicted aspects of the restoration of granule material in a gland which has undergone previous stimulation are given by Fig. 2. The calculations were made using constants determined in a typical experiment, and so are believed to have a general significance.

It will be noted from Fig. 2a that for the value of  $\beta$  used, there is a 96% recovery within 4 hr. after the depletion of the store of granule material to 20% of its original value. In our experiments the calculated depletion has rarely been greater than 32% of the original value.

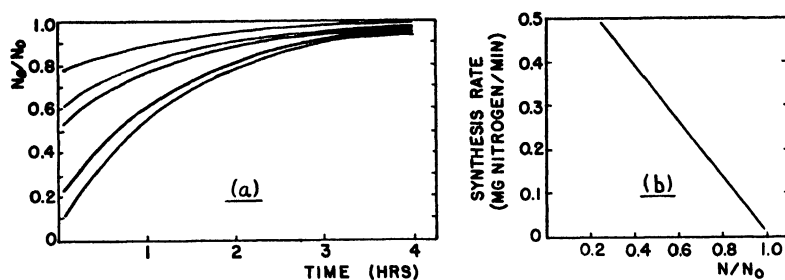


FIG. 2. Some calculated features of the synthesis of protein material. (a) Progress of the restoration of granule material in a resting gland after various degrees of depletion (Equation 9).  $N_g/N_0$  denotes the amount of granule material in the gland expressed as a fraction of the amount in the "rested" gland. The time is reckoned from the start of the rest period. (b) Relation between the rate of synthesis and the amount of granule material present in the gland (Equation 2). The curves of (a) and (b) were calculated with the constants determined for Experiment 6, Table II, viz.,  $100\beta = 1.20/\text{min.}$ , and  $N_0 = 5.4$  mg. of protein nitrogen.

### Comparison of Theory and Experiment

Tables I, II, and III compare predicted and observed protein outputs for three different types of experiment. The mode of representation is as follows. The number of each sample of secretion is given in the first column, and  $T$  denotes the time of collection (in minutes from the beginning of the experiment); "Inj." denotes the amount of secretin injected per 5 min. (in mg.);  $\delta N$  denotes the output of protein material in milligrams of nitrogen, in each sample.

TABLE I

AN UNINTERRUPTED SERIES OF SAMPLES OBTAINED UNDER A CONSTANT RATE OF ADMINISTRATION OF SECRETIN

Experiment 5 (see (4, Table I)): Dog, 15.4 kg.

Inj. = 6.1       $100\beta = 0.44$        $N_0 = 107$  mg.       $100k\sigma = 1.06$

Sample No.	$T$	$\delta N$		Sample No.	$T$	$\delta N$	
		Calc.	Obs.			Calc.	Obs.
1	30	29.4	29.5	5	150	13.2	13.8
2	60	22.4	22.5	6	180	12.1	12.0
3	90	17.7	18.4	7	210	11.2	11.2
4	120	15.1	15.8	8	240	10.9	10.6

It may be seen from Tables I to III that the agreement between the calculated and observed values is remarkably good, although the experimental conditions varied over a wide range. In eight experiments the average deviation of the calculated from the observed outputs was only 4.5%. In

TABLE II  
AN UNINTERRUPTED SERIES OF SAMPLES OBTAINED UNDER VARIED RATES OF ADMINISTRATION OF SECRETIN

Experiment 6 (see (4, Table II)): Dog, 7.5 kg.

$N_0 = 54$  mg.;  $100\beta = 1.20$

Sample No.	$T$	Inj.	$\delta N$		$100 k\sigma$	$100k\sigma/\text{Inj.}$
			Calc.	Obs.		
1	30	6.0	14.3	14.0	1.00	0.167
2	60	6.0	11.5	12.6		
3	90	6.0	10.3	10.0		
4	120	6.0	9.4	8.9		
5	150	6.0	9.2	9.0	0.28	0.186
6*	160	1.5	(0.88)	(1.11)		
7+8	220	1.5	6.10	5.99		
9*	230	6.0	(4.2)	(2.5)		
10	260	6.0	11.4	12.4	1.10	0.183
11	290	6.0	10.4	11.8		
12*	305	1.5	(1.33)	(2.18)	0.28	0.186
13+14	365	1.5	6.18	5.81		
15*	375	6.0	(4.22)	(4.33)		
16	405	6.0	11.5	11.8		
17	435	6.0	10.4	10.6	1.10	0.183
18	465	6.0	9.7	10.1		
19	495	6.0	9.5	9.1		
20*	510	1.0	(0.72)	(1.42)		
21+22	570	1.0	3.6	3.60	0.16	0.160
23	600	4.0	6.7	7.10		
24	630	4.0	6.3	5.93	0.55	0.137
25	660	4.0	6.3	6.35		
26	690	4.0	6.2	6.35		

\* These samples were taken to clear the ducts of secretion due to the previous stimulation. Because they contain some secretion due to the previous stimulation, their observed  $\delta N$  values are expected to be higher than calculated on changing from a high to a lower rate of secretin administration, and lower than calculated on changing from a low to a higher rate. They are of little use in comparison of theory and experiment.

TABLE III

UNINTERRUPTED SERIES OF SAMPLES OBTAINED BEFORE AND AFTER A 2-HOUR PERIOD OF "REST";  
CONSTANT RATE OF ADMINISTRATION OF SECRETIN

Experiment 7 (see (4, Table III)): Dog, 17.5 kg.

Inj. = 8.0       $100\beta = 0.53$        $N_0 = 85.3$  mg.       $100k\sigma = 1.19$

Sample No.	$T$	$\delta N$		Sample No.	$T$	$\delta N$	
		Calc.	Obs.			Calc.	Obs.
3	140*	11.1	11.2	7	380	17.3	18.4
4	170	9.8	9.9	8	410	14.2	14.5
5	200	9.7	9.7	9	440	12.2	13.7
6	230	9.1	9.4	10	470	11.1	11.4
—	230–350	Rest period†		11	500	10.4	11.0

\* This sample was taken between 110 and 140 min. from the start of the experiment.

† The calculated amount of granule material, as nitrogen, present in the gland immediately after collection of the 6th sample was 27 mg.; at the end of the rest period it was 54 mg. Hence the increased protein output in the samples of the latter group.

one experiment in which the technique was not quite satisfactory, one deviation of 26% and one of 28% occurred. Aside from these two samples, the distribution of the deviations of calculated from observed values is given by

Deviation (%)	<5	5 — 10	10 — 15	>15
Number of samples	46	18	7	0

It is to be emphasized that without exception the predicted characteristic features of the protein output behaviour are observed. These features, as listed in the preceding section, are illustrated by the following experiments: point (i) by the first group of samples in Experiments 5, 6, and 7 (Tables I, II, and III); point (ii) by Experiment 6 (Table II); point (iv) by Experiment 6; and point (v) by Experiment 7 (Table III). Point (iii) is illustrated by an experiment (No. 9) that is not given in the tables. In Experiment 9, eight 30-min. samples were obtained under a constant rate of secretin administration, and then eight more were obtained with one-third the rate of administration. In the latter group the observed protein outputs, in mg. of nitrogen, were respectively 2.2, 2.6, 2.8, 2.8, 2.9, 2.8, 2.8, 3.0, while the corresponding calculated values were, 2.4, 2.6, 2.8, 2.8, 2.8, 2.9, 3.0, and 3.0. These figures clearly show the gradual rise in the protein output as predicted in point (iii). It may be added that the theory was used to design several of the experiments to test points predicted by it.

A fundamental point in the comparison of theory and experiment is that  $k\sigma$  is observed to be closely proportional to the rate of administration of secretin, except toward the end of long experiments (see Table II). This finding establishes the validity of the definition of  $\sigma$  in Equation (1). The fact that the proportionality fails towards the end of very long experiments is to be expected, and will be discussed in the following section.

## Discussion

The fact that the theoretical expressions quantitatively describe all the observed characteristic features of the protein outputs in the samples of widely varied experiments, is strong evidence that the basic assumptions of the theory are good approximations. The theory gives the following picture of the secretory processes as brought about by administration of secretin. The influx of secretin into the gland causes certain definite, and probably largely independent, processes to take place. (a) The mechanism responsible for the secretion of water is set in operation at a rate dependent in some way on the amount of secretin present in the gland. No attempt has been made by us to investigate the nature of this mechanism. In the present theory, the flow of water serves simply to carry the products of the secretion reaction into the glandular ducts, and of course to remove such crystalloids as may transfuse through the glandular membranes. (b) A chain of chemical reactions, represented by a definite velocity equation and probably irreversible, is set up in the cells. The first step involves secretin and the last results in

the transformation of granule material to a form or forms readily carried out of the cells by the flow of water. (c) As the amount of granule material in the cells is diminished by the secretion reaction of (b), a reversible chemical reaction is set up, resulting in the formation of more granule material. On this view, synthesis takes place when one of the components entering into the synthesis reaction (*i.e.*, granule material) is reduced to an amount below that existing in the equilibrium state. (d) Whether the character of the cell membranes is affected by changes in the intensity of the stimulus is uncertain, since the ease of passage of such crystalloids as sodium and potassium is so great that it might be thought to be unaffected by changes of a moderate magnitude in the membranes (*cf.*, (4) ).

It is desirable to point out that the theory that describes the protein output in the submaxillary saliva of the cat (5) is only a special case of the more general theory here applied to the pancreas of the dog. The general nature of the processes is the same in both glands, although the chemical reactions involved are of course not identical. As previously pointed out, the rate of synthesis in the submaxillary gland is normally very small, but in the pancreas it is comparatively great. This may be due in part to the difference in the properties of the membranes of the two glands (*i.e.*, to the difference in rate of transfusion of substances required for synthesis).

In comparing calculated and observed protein outputs, it was assumed that the amount of secretin  $\sigma$  present in the gland remained constant during periods characterized by a constant rate of administration of secretin. As secretin injections were made at 5-min. intervals, however, the amount of secretin in the gland must have undergone fluctuations over a 30-min. period. In such a case the observed protein outputs may be well expressed by the equations, if  $\sigma$  represents the average amount of secretin in the gland during the 30-min. period. This is true only if the interval between successive secretin injections is small. It was shown to be so, by calculations based on the observed rate of diminution of water flow after stopping the administration of secretin (4, Table III). It seems reasonable to suppose that the average amount of secretin present in the gland during a 30-min. period is directly proportional to the amount injected into the femoral vein, although the proportionality constant may be expected to vary from animal to animal. This consideration is important in interpreting the  $\frac{k\sigma}{\text{Inj.}}$  ratios, which are found to be essentially constant for any animal, except toward the end of long experiments.

The following remarks may be made concerning the synthesis reaction. The fact that similar results are obtained whether the parasympathetic nerves are paralysed by atropine or not, indicates that synthesis during periods of secretory activity provoked by secretin administration is not dependent on the parasympathetic nervous system. Nor is it influenced by the presence of secretin in the gland, as indicated by the constancy of  $\beta$  in experiments in which the rate of secretin administration was varied, and by the fact that

synthesis goes on in a regular way when no secretin is administered (Table III). According to the considerations of the section on formulation of the theory, the rate at which synthesis proceeds depends on the amounts of granule material and of certain unknown substances (the  $D$ ,  $E$ ,  $F$  of (2a)) present in the gland. Equation (2) with  $N_0$  constant led to a remarkably good description of the experimental data; a comparison of (2) with the more general equation (2a) shows that  $N_0$  of (2) stands for  $(DEF \dots / D_0 E_0 F_0 \dots) N_0$ . Hence the implication is that  $DEF \dots / D_0 E_0 F_0 \dots$  remained essentially constant, *i.e.*, the amounts of substances required for synthesis present in the gland were not appreciably depleted during an experiment. It seems reasonable to suppose therefore that these substances were supplied continuously to the gland from an external source (*e.g.*, the blood stream), since if they originated from stores within the gland a more rapid depletion would be expected. The difference in the  $N_0$  values found for different dogs is readily understandable, because  $N_0$  will depend on the size of the gland and on the amounts of the substances  $D$ ,  $E$ ,  $F$ , . . . present in the system. It is interesting to note that  $N_0$  is roughly determined by the weight of the dog, as might be expected (Tables I, II, and III). The  $\beta$  values vary somewhat from animal to animal, but why this should be so is not yet clear.

The following remarks may be made concerning the secretion reaction. If transformation of granule material to the secreted form or forms took place as the result of a direct reaction between secretin and granule material, no appreciable variation of the  $k\sigma/\text{Inj.}$  ratio should be expected throughout an experiment. While the ratio remains constant for a long time, it is observed to decrease gradually toward the end of long experiments (*cf.*, Table II). If the secretion reaction is a chain reaction, however, such a decrease may be expected on the following basis. According to (1a), the rate at which the secretion reaction proceeds depends on the amount of granule material, of secretin, and of certain other substances,  $A_1$ ,  $A_2$ , . . .  $B_1$ ,  $B_2$ , . . . , present in the gland. The calculations given in the tables were made with the simplified form, Equation (1). In Equation (1),  $k$  really represents  $k^1(A_1 A_2 \dots)(B_1 B_2 \dots)$ , so that a decrease in the amounts of the substances  $A_1$ ,  $A_2$ ,  $B_1$ ,  $B_2$ , present in the gland, results in a decrease in the value of  $k$  of (1), and so also of  $k\sigma$ . The observed decrease in the  $k\sigma/\text{Inj.}$  ratio is therefore attributed to a decrease in the amounts in the gland of certain substances necessary for the secretion reaction. The fact that the amounts of these substances remain essentially constant for so long a time (more than 8 hr. in Experiment 6, Table II) is considered to indicate that they are continuously supplied to the gland from an external source (*e.g.*, the blood stream).

It should be emphasized that the validity of the assumptions underlying the theory cannot be regarded as definitely established by the close agreement between calculated and observed protein outputs, nor are the interpretations given in this section necessarily unique. We have chosen the simplest assumptions and interpretations consistent with the data and with other known facts,

but it is possible that the gland behaves in a more complicated manner. If this is so, the operation of the glandular mechanisms must still be closely described by the given equations, since these fit the observed data over a wide range of experimental conditions. The chief function of the theory, and of the interpretations based on it, lies in their power to suggest new problems in connection with the secretory processes.

### Acknowledgments

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## INVESTIGATIONS ON TRICHINOSIS IN CANADA

### II. A FURTHER SURVEY OF THE INCIDENCE OF *TRICHINELLA SPIRALIS* IN HOGS IN EASTERN CANADA<sup>1</sup>

By THOMAS W. M. CAMERON<sup>2</sup>

#### Abstract

During 1937 and 1938, 2,000 hogs from eastern Canada were examined for cysts of *Trichina*. Fifteen or 0.75% were infected.

During the summer of 1938, the survey recorded in 1937 (1) was continued. The same technique of receiving the samples and of examining them at the Institute of Parasitology was followed, and the same general sources of supply were used. All samples were selected at random and no selection was made except that samples were sent only from hogs of which the district of origin was known. Ten grams of muscle was digested as before, while part of the remainder was examined in the compressorium.

During this season, 1,271 samples were received, making a total of 2,000 for the two seasons. Although 15 positive samples were received in 1937, there were none in 1938. To check the technique employed, known positive samples were examined in an identical manner at intervals throughout the season. In all these cases, the results were positive, confirming the efficiency of the technique. It may be taken, therefore, that the pork samples received from the abattoirs actually were negative. The reasons for the completely negative results this year are not obvious.

An analysis of the examination for the two years (Table I) accordingly shows an incidence of 0.75%, a figure considerably under that suggested in the early part of the survey.

The Bureau of Animal Industry in the past four years has recorded the results of examinations of hogs in the United States as follows:

1935	1,973 garbage-fed hogs	4.8%	infected
	2,146 grain-fed hogs	1.5%	infected
1936	2,341 garbage-fed hogs	5.0%	infected
	4,740 grain-fed hogs	1.0%	infected

<sup>1</sup> Manuscript received April 18, 1939.

Contribution from the Institute of Parasitology, McGill University, Macdonald College, Que., with financial assistance from the National Research Council of Canada.

<sup>2</sup> Professor, McGill University; and Director, Institute of Parasitology, Macdonald College, Que., Canada.

1937	3,876 garbage-fed hogs	3.4%	infected
	1,860 cooked garbage-fed hogs	0.59%	infected
	1,535 no garbage-fed hogs	0.32%	infected
1938	2,847 garbage-fed hogs	10.0%	infected
	3,799 grain-fed hogs	1.0%	infected

TABLE I

INCIDENCE OF TRICHINA CYSTS IN HOGS EXAMINED IN 1937 AND 1938

Province	No. of examinations			Positive	
	1937	1938	Total	No.	%
Saskatchewan	7	0	7	0	0
Manitoba	299	467	766	5	0.65
Ontario	146	405	551	3	0.54
Quebec	226	274	500	6	1.2
New Brunswick	40	46	86	0	0
Nova Scotia	5	21	26	0	0
Prince Edward Island	6	39	45	1	2.2
Unlabelled	—	19	19	0	0
Total	729	1,271	2,000	15	0.75

The percentage infections vary considerably in these samples. The general average is 3.1% but when the 11,000 hogs fed on raw garbage are contrasted with the 14,000 hogs not so fed, it will be noted that 5.85% of garbage-fed hogs are infected, contrasted with 1.0% of the hogs fed no raw garbage. As the feeding of raw garbage to hogs is prohibited by law in Canada, the latter figures only are comparable with our results. The results are quite similar.

There seems little doubt that the majority of pigs infected with trichinosis in North America owe this infection to uncooked garbage. Consequently the majority of cases of human trichinosis must be indirectly due to this cause also. This conclusion is strengthened by the fact that (where such comparisons have been made) garbage infections are heavier than the others.

However, it would seem that there remains a residue of about 1% which is *not* due to garbage and the origin of which is still obscure.

### Acknowledgment

Again the author has to thank the Veterinary Director General of Canada for his generous co-operation in this investigation, as well as Drs. W. H. Bright, Ed. Grandmaison, J. G. MacDonald, D. T. McLellan, J. Naismith, G. A. Rose, and D. C. Tennant. Without this, this investigation would have been impossible. The actual examination of the samples in the laboratory was carried out under the author's supervision by Mr. Angus MacMillan, who was employed for this project with the aid of a special grant from the National Research Council of Canada.

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## PARASITES OF FRESHWATER FISH

### I. INTERNAL TREMATODES OF COMMERCIAL FISH IN THE CENTRAL ST. LAWRENCE WATERSHED<sup>1</sup>

By L. L. Lyster<sup>2</sup>

#### Abstract

Sixteen species of trematodes from ten species of fish are discussed. *Ptychogonimus fontanus* sp. nov. from *Perca flavescens* is described.

#### Introduction

Though Stafford (16) studied parasites of hosts from eastern Canada, and many American authors have dealt with the subject of fish parasites in comparable climatic regions, internal parasitism in freshwater fish in Quebec has never been investigated exhaustively. The series that this paper introduces is planned to add to existing knowledge of distribution, and to host and parasite records.

The fish dealt with here are those supplying the inland fisheries of the province. The material came partly from the spirit-preserved collection of the Institute of Parasitology, partly through fish merchants in Montreal, and partly from fish caught for the purpose during the summers of 1937 and 1938. Most of the hosts reported were taken at the confluence of the Ottawa and St. Lawrence Rivers or in Lake St. Louis. A host that does not appear in our lists was also examined: *i.e.*, *Cyprinus carpio*; examination of 200 viscera showed no internal trematodes, though freedom from such parasites does not seem an anticipated condition for these fish. Our collections were made in October 1937, and some seasonal factor may have been involved.

The systematic names of fish appearing here are those used by Hubbs (9).

#### Species Recovered

FAMILY BUCEPHALIDAE Poche, 1907

*Bucephalopsis pusilla* Stafford, 1904

Host: *Stizostedion vitreum*

The specimens were slightly larger than those described by Van Cleave and Mueller (18), being about  $0.25 \times 0.8$  mm. when mounted, but other features were typical: ventral sucker  $0.040 \times 0.048$  mm. and cephalic sucker (exserted in some slides and retracted in others), about 0.112 mm. in both diameters, gastric element  $0.16 \times 0.22$  mm., median and dorsal to the ventral sucker through which the short oesophagus opens; ovary ventral to it and displaced to the side; oviduct loops anteriorly and joins genital pore in the

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Contribution from the Institute of Parasitology, McGill University, Macdonald College, with financial assistance from the National Research Council, awarded through the National Committee on Fish Culture.

<sup>2</sup> Graduate assistant.

posterior third; testes spherical with margins entire, the anterior medial, caudad to the ovary and close to or in contact with it, the posterior lying obliquely; cirrus sac a prominent tubular structure lying along the right lateral margin of testes and opening posteriorly into the common genital pore; vitellaria composed of distinct bead-like structures antero-lateral to intestine joined by prominent tubes emptying into a common organ near anterior testis; the medio-terminal excretory pore is easily seen.

FAMILY GORGODERIIDAE Looss, 1906

Two forms were taken, from pike and a muskellunge respectively, that appeared to be typical members of the genus *Phyllodistomum* as discussed by Pearse (13) and classified by Holl (5), but were collected from the alimentary tract. As this genus is not normally an intestine dweller, we must conclude that these individuals are (i) ectopic forms or (ii) parasites of food-fishes of these predators.

*Phyllodistomum superbum* Stafford, 1904

Host: *Esox lucius*

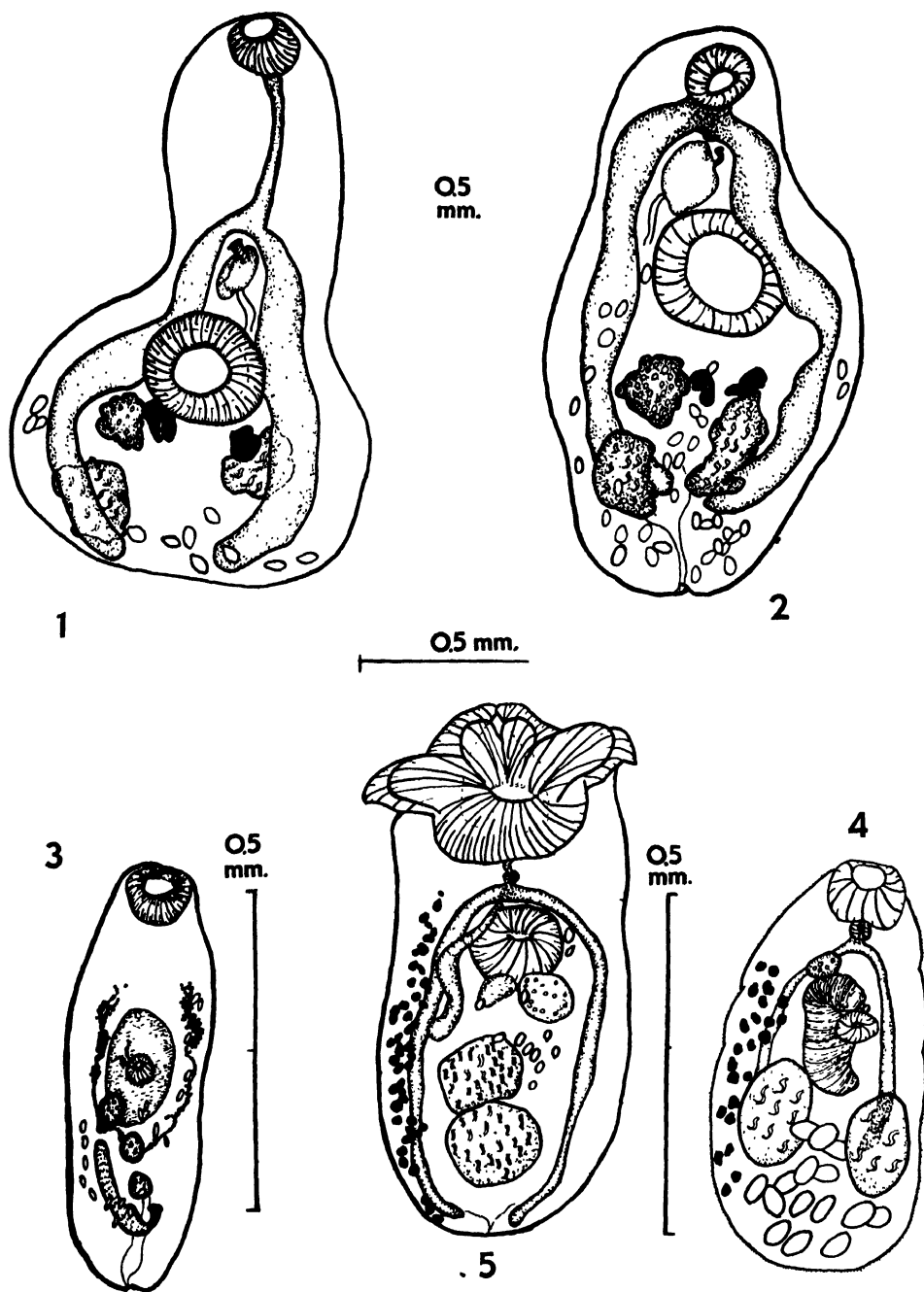
In this species the body outline is regular, widening to just behind the acetabulum, then narrowing slightly to the rounded tip; it is about 1.0 mm. in length and slightly more than 0.5 mm. at the widest point. Papillae-like corrugations occur in the cervical region lateral and anterior to the acetabulum, measuring about  $0.03 \times 0.001$  mm. Oral sucker about 0.13 mm. in diameter, the cephalic region at this point measuring 0.30 mm.; no pharynx present and oesophagus very short or absent; wide sac-like crura extend posterior to testes; acetabulum prominent (approximately 0.2 mm. in diameter), lying in a medial central position; the large bladder-like cirrus sac, opening through the small post-crural genital pore, is anterior to the acetabulum.

The lobed ovary lies well posterior to the acetabulum and displaced to the left. The anterior testis is transversely in line with it and the second testis directly posterior to it. Both are lobate and larger than the ovary. Vitelline glands are lobate pyriform structures close to anterior margins of the ovary and first testis. The uterus coils to the posterior end of the body, prominent intra-uterine eggs  $0.023\text{--}0.027 \times 0.033\text{--}0.040$  mm. in size being present. The excretory pore opens into a distinct median notch.

*Phyllodistomum staffordi* Pearse, 1924

Host: *Esox masquinongy*

A single specimen was taken from the muskellunge. It is characterized by: narrow forebody, flat leaf-like hindbody, narrowest at anterior margin of acetabulum (0.37 mm.) and widest in region of anterior testis (0.72 mm.), about 0.89 mm. in length; acetabulum larger than oral sucker; short oesophagus present, but no pharynx; cirrus sac and genital pore as in *superbum*; testes, ovary, and vitellaria very much as in *superbum* but rotated and widely separated laterally; posterior testis close to body margin; intra-uterine eggs measured  $0.023\text{--}0.030 \times 0.033$  mm.; no distinct median notch.



FIGS. 1-5. 1. *Phyllodistomum staffordi* from *Esox masquinongy*. 2. *Phyllodistomum superbum* from *Esox lucius*. 3. *Bucephalopsis pusilla* from *Stizostedion vitreum*. 4. *Vielosoma parvum* from *Ictalurus punctatus*. 5. *Crepidostomum cornutum* from *Ambloplites rupestris*.

## FAMILY PLAGIORCHIIDAE Lühe, 1901

*Vietosoma parvum* Van Cleave & Mueller, 1932Host: *Ictalurus punctatus*

A single minute fluke from the stomach of a channel catfish belonged to this species. Little can be added from this specimen to the authors' description. The only variation was in the extent of the seminal vesicle, which is much larger than shown by Van Cleave and Mueller (18). It extends from a point level with the anterior margin of the rearmost testis dorsal to the acetabulum and well anterior to it, then bends backwards and down to the ventral surface, where it joins the genital pore on the left anterior margin of the ventral sucker. A single small ovoidal structure ventral to the right crura and anterior to the vesicle seemed to be the ovary. Whether further follicles were obscured by the vesicle or whether this represented the entire structure is in doubt, although appearances are in favour of the latter view. Two smooth-margined testes are conspicuous, slightly oblique to each other. The uterus reaches the posterior limit of the body, where an egg sac is formed. No prepharynx could be seen. The suckers, crura, and vitellaria all follow closely the original description, and the shape and size and superficial appearance are so nearly identical with published descriptions of *parvum*, that, in spite of what seem considerable variations in the genital complex, the specimen must be assigned to this species.

## FAMILY ALLOCREADIIDAE Looss, 1900

*Crepidostomum lintoni* Pratt, 1901 (in Linton)Host: *Acipenser fulvescens*

This species, the only trematode found in the sturgeon, was present in great numbers. This is a small lanceolate fluke, usually under 1 mm. in length and about 0.3 mm. wide, with prominent oral papillae and a large oral sucker. Prepharynx absent, pharynx ovoidal, and approximately one-fifth the oral sucker in longitudinal diameter followed by distinct oesophagus; crura widen shortly after caecal fork, passing within short distance of the end of the body; genital pore median, close behind caecal fork. Ventral sucker in anterior half of body, or medial, and smaller than oral sucker. Ovary pear-shaped and lobed left of and well behind acetabulum, testes slightly lobed, close together, tandem or somewhat oblique and posterior to ovary. Vitellaria rise anterior to pharynx and lie laterally or dorsally along body, meeting at terminal mid-line. Broad flat muscular cirrus sac reaches posterior margin of acetabulum and contains prominent cirrus; uterus reaches anterior to testes or less.

*Crepidostomum cornutum* Osborn, 1903Hosts: *Ambloplites rupestris**Micropterus dolomieu*

These are medium to small forms, roughly rectangular in outline, and length two to six times the width. Oral sucker large, 3 to 4 times the acetabulum,

and equipped with prominent lateral papillae; oesophagus thin-walled; cirrus sac prominent, S-shaped and reaching to ovary. Genital pore and acetabulum close behind intestinal division; ovary ovoidal or spherical, sometimes projecting dorsal to acetabulum, always close to it. These organs lie within first half of the body. Two spherical testes occupy remainder. Testes usually very prominent but may be greatly reduced or even absent. Uterus turns back as far as posterior testis or less and passes dorsally or right of acetabulum. Caecae rise close behind pharynx and extend to posterior region of body; vitellaria lie laterally from pharyngeal area to end of body.

*Crepidostomum cooperi* Hopkins, 1931

Hosts: *Micropterus dolomieu*

*Perca flavescens*

This form shows variation in gross anatomy within the same host. Some are roughly diamond-shaped, widest in the region of the ovary with an anterior expansion to accommodate the oral sucker, 0.2–0.3 mm. in diameter with six papillae of relatively uniform size, others are rectangular.

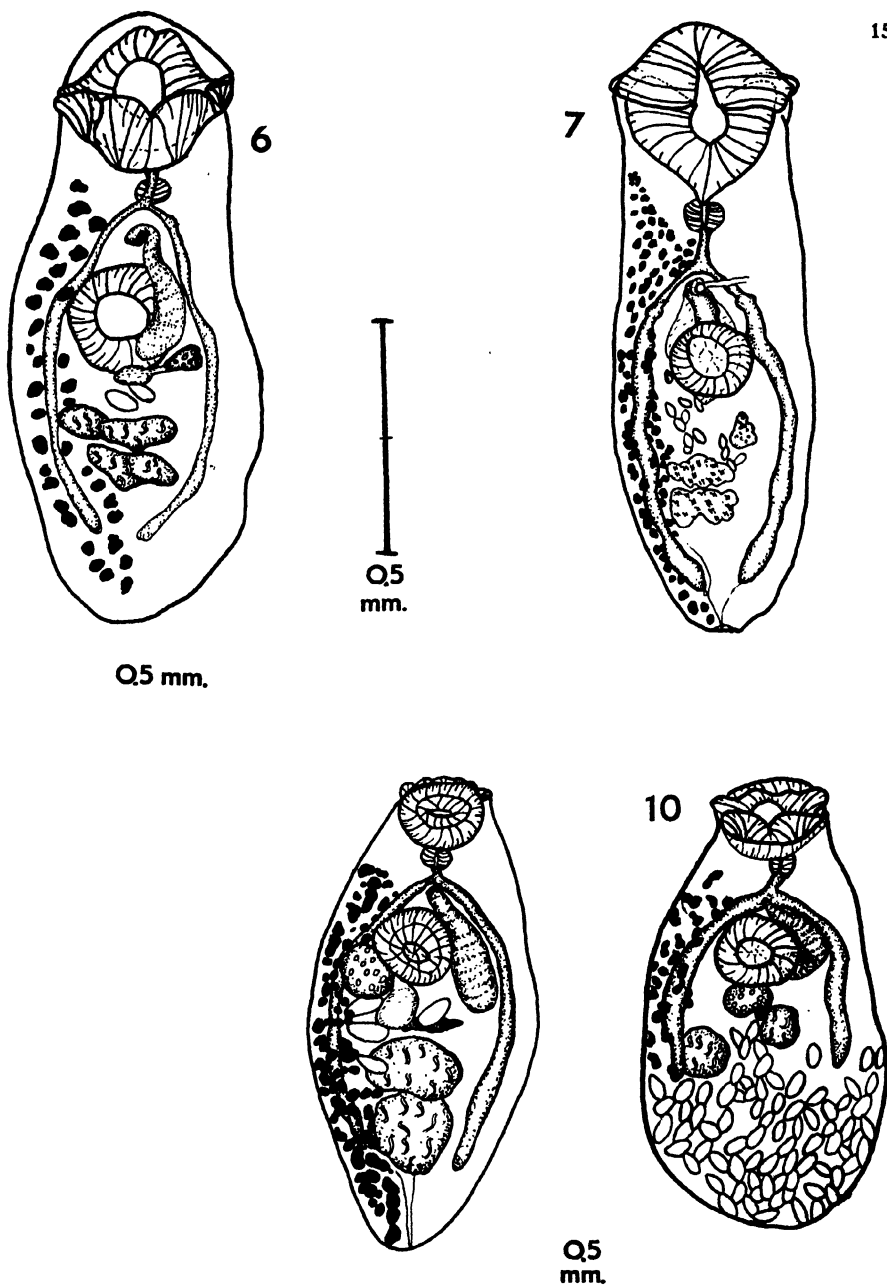
The oral sucker is followed by pharynx, medium in size, and thin-walled oesophagus. Caecae may terminate at about the third quarter of the body length, or continue to within a short distance of posterior limit. Ventral sucker about equal in size or larger than the oral sucker except when contraction makes the former appear smaller; it is about its own diameter behind the pharynx, and the oesophagus may be slightly looped to accommodate it. It is often slightly displaced laterally. Genital pore lies close between acetabulum and crural division. Cirrus sac extends posteriorly, dorsal and sometimes lateral to ventral sucker as far as ovary at least. The ovary lies close to acetabulum and slightly to the right, overlapping it in many cases. A seminal receptacle is prominent medial to it.

These organs occupy about half the body. Two large prominent testes, invariably present, regularly spherical or only slightly lobed in outline, are tandem and fill most of remaining portion. Vitelline glands extend along the lateral margins from oesophagus to posterior end of body, often filling in area behind testes. Uterus, containing a few large ovoidal eggs, extends posteriorly to anterior testes. Distinct specimens in author's collection conform to Hopkins' (6) description of *C. ambloplitis*, others to that of *C. cooperi*. However, intermediate forms make differentiation of these species impossible and the terms must be considered synonymous. This supports the assertion made by Van Cleave and Mueller (18), who described the single species *C. solidum* and later noted the synonymy of their species and those of Hopkins. The name *cooperi*, therefore, has been retained as the valid species indication, with *ambloplitis* and *solidum* reduced to synonymy.

*Crepidostomum ictaluri* Surber, 1928

Host: *Ictalurus punctatus*

These are typically short thick forms, slightly lanceolate, about 0.96–1.2 × 0.37–0.5 mm.; large hexapapillate oral sucker, short prepharynx, barrel-



FIGS. 6 - 10. 6. *Crepidostomum ictaluri* from *Ictalurus punctatus*. 7. *Crepidostomum lintoni* from *Acipenser fulvescens*. 8. *Crepidostomum brevitetellum* from *Anguilla rostrata*. 9. *Crepidostomum cooperi* from *Perca flavescens*. 10. *Bunodera sacculata* from *Perca flavescens*.

shaped pharynx, and short oesophagus present. Caecae extend to posterior part of the body. Acetabulum at least equal to and sometimes larger than oral sucker, occupies central position in body some distance behind crural bifurcation. Genital pore just behind the caecal fork. Cirrus sac extends posteriorly, dorsal to the acetabulum as far as caudal margin of ovary; ovary pear-shaped and situated postero-lateral to ventral sucker; seminal receptacle medial and dorsal. The large testes are markedly lobed to the extent that they may appear to be four distinct organs. However, in the slides that were examined the bi-testicular condition was very clear and the division of the organs recognizable as a medial constriction, rather than forming the "four testes" described by Walz (20). In several very small and apparently young forms the posterior quarter was medially free of organs, probably to be filled by the developing adolescariâ, while the two immature testes, when present, were entire, removing any doubt as to the fundamental nature of these organs. The factor on which Surber (17) based his genus *Megalogonia* is, therefore, considered here as of specific significance only.

*Crepidostomum brevivitellum* Hopkins, 1934 .

HOST: *Anguilla rostrata*

These are elongate, narrow flukes, uniform in width throughout or tapering slightly from region of intestinal bifurcation; oral sucker with simple papillae, lateral members slightly longer than leaf-like dorsal ones. Pharynx ovoidal or globular. Oesophagus conspicuous. Intestinal crura extend behind posterior testis, but not to extremity of body. Acetabulum slightly ovoid, smaller than complete oral sucker. Ovary margin entire, globular, medial, well behind acetabulum. Prominent seminal receptacle globular or ovoid to right and near ovary and dorsal to it, usually in contact with ovary and anterior to first testis by, at least, its own diameter. Two testes, margin entire, globular, medial and tandem, anterior to tip of body by at least one diameter. Anterior testis well in front of posterior. Vitellaria occupy lateral area from a point in line with posterior margin of acetabulum to near tip of body, surrounding crura and extending into inter-crural area dorsally and meeting at midline behind testes. Cirrus sac thin-walled and curved; common genital opening lies close to anterior margin of acetabulum. Uterus fills space between anterior testis and acetabulum, containing 23 to 100 eggs.

These specimens have a shorter cirrus sac than is described for the species. It rarely passes much beyond the acetabulum. In this respect they are similar to the *C. latum* of Pigulewsky (14), but in extent of the excretory bladder and relative positions of the seminal vesicle and ovary they are identical with *C. brevivitellum*.

In some organs in the present material, measurements vary from those of the original description. As this is the first record confirming Hopkin's (8) report of the species, measurements are listed here in detail.

Oral sucker	0.26 - 0.3 mm. diameter
Pharynx	0.11 - 0.14 mm. longitudinal diameter

Oesophagus	0.18 – 0.2 mm. lateral diameter
Acetabulum	0.19 – 0.22 mm. longitudinal diameter
	0.22 – 0.26 mm. lateral diameter
Ovary	0.09 – 0.11 mm. diameter
Receptacle	0.07 – 0.15 mm. greatest diameter
	0.26 – 0.34 mm. length
Anterior testis	0.24 – 0.3 mm. longitudinal diameter
	0.26 – 0.34 mm. lateral diameter
Posterior testis	0.22 – 0.26 mm. longitudinal diameter
	0.32 – 0.37 mm. lateral diameter
Eggs	0.042 mm. diameter
	0.056 – 0.07 mm. length
Vitelline follicles	0.03 × 0.04 – 0.07 mm.
Body	0.41 – 0.5 mm. breadth
	2.7 – 3.12 mm. length

*Bunodera sacculata* Van Cleave & Mueller, 1932

Host: *Perca flavescens*

These small sac-like flukes measure approximately  $1.0 \times 0.5$  mm. Oral sucker equipped with six non-prominent papillae; pharynx and small, but extended, oesophagus present; intestinal crura extending to region of posterior testes; vitellaria occupying very much the same position; the genital pore medial and ventral to caecal fork; short, broad cirrus sac extends lateral and dorsal to acetabulum and terminates only slightly, if at all, posterior to it.

Ventral sucker (lying close behind the crural fork in a medial or slightly laevad position) approximately equal in size to the oral sucker; smooth-margined ovary more or less medial and close to acetabulum or overlapping its posterior area. Anterior testis usually lies near right posterior margin of the ovary with medial seminal receptacle; posterior testis near left margin of body widely separated from other genital organs. The remaining and widest part of the body is taken up by the uterus, filled with numbers of prominent eggs. Lateral examination of a mature specimen shows that the increase in size is carried ventrally as well as transversely to make the posterior part of the animal an egg sac which increases its capacity as the uterus fills.

5. FAMILY HETEROPHYIDAE, Odhner, 1914

*Centrovarium lobotes* MacCallum

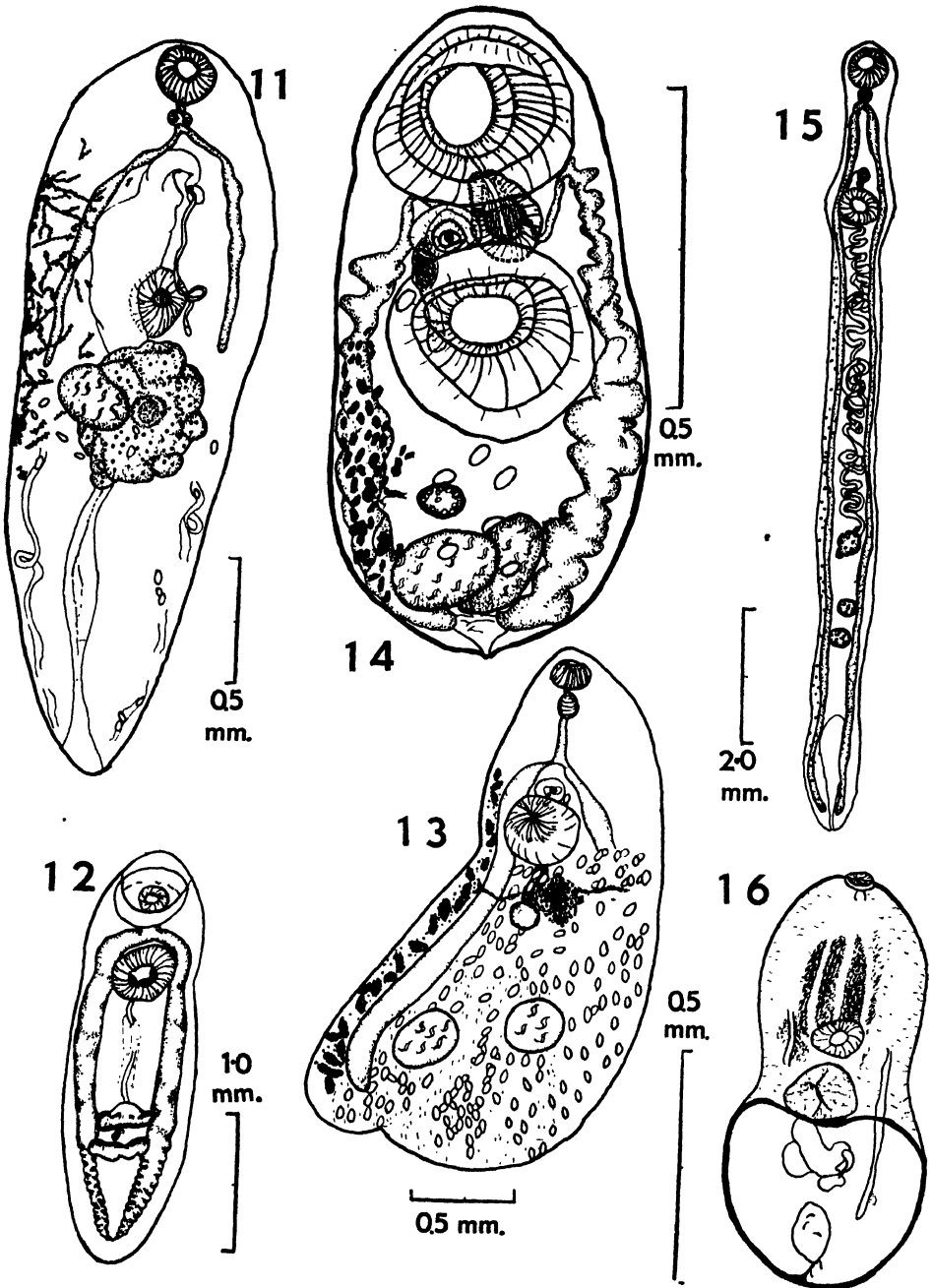
Hosts: *Esox lucius*

*Ameiurus nebulosus*

*Ambloplites rupestris*

*Stizostedion vitreum*

Adult specimens were taken from the first three hosts listed and fragments were collected from the last. The degeneration that is characteristic of adults of these flukes was marked in all specimens. In a small individual from the



FIGS. 11 - 16. 11. *Centrovarium lobotes* from *Stizostedion vitreum* (oesophagus turned ventral). 12. *Clinostomum complanatum* (larval). 13. *Acelodextra amiura* from *Ameiurus nebulosus*. 14. *Ptychogonimus fontanus* from *Perca flavescens*. 15. *Azygia longa* from *Esox lucius*. 16. *Posthodiplostomum minimum* (larval) from *Ambloplites rupestris*.

pickerel the ovary had retained its typical rosetiform appearance. The fluke is regularly oval in outline, tapering slightly at both ends. The oral sucker is of medium to small size; prepharynx, short pharynx, and oesophagus present; caeca extend posteriorly to a point just cephalad of the ovary; vitellaria of loosely arranged follicles lateral, but may meet in midline, from anterior extremity in line with crural fork they reach only slightly posterior to the ovary. Ventral sucker, roughly equal in size to the oral sucker, about twice its diameter, behind crural furcation. Ovary very prominent, at least twice acetabulum in diameter; it is circular and very lobate in outline with a central dense portion, and equipped with a very prominent seminal vesicle that extends from region of the genital pore laterally and well behind the acetabulum. Uterus large, convoluted, filling caudal half of the body with numerous eggs. Large dorsal excretory sac usually prominent. In smaller forms, no testes observed; where present, anterior testis close to or directly dorsal to ovary, smoothly margined and comparable in size to acetabulum. A second testis was never present. In large specimens from both major hosts the vitellaria were often absent and the ovary much reduced.

*Acetodextra amiura* (Stafford 1900) Pearse, 1924

Host: *Ameiurus nebulosus*

Many trematodes referable to the genus *Acetodextra* were taken from the swim bladder of common bullheads. This parasite has been the cause of considerable difficulty with experimental bullheads in this laboratory, where it has caused the death of the fish whenever present in any numbers. It seems to be limited entirely to the bullhead for adult development, no doubt owing to the external opening of the swim bladder of this fish in adult life, and, its pathogenicity established, an economic application is apparent whereby the bullhead population might be limited in hatchery water, game-fish pools, spawning beds, etc.

In spite of its somewhat unique organization, it is included here as a Heterophyid on the authority of Mueller and Van Cleave (12).

The flukes are widest terminally, tapering to the oral sucker; body  $0.19 \times 0.23$  mm. in greatest measurement, with very delicate non-spined cuticle. Most organs were obscured by the operculate eggs,  $0.004 \times 0.006$  mm., bearing a small terminal protruberance, and many of the details noted were found by dissection (measurements given here are for a single specimen). Ovary a composite organ of many small follicles in a petalose arrangement; two testes in rear portion of body, set in a deeply staining tissue that fills the latter part of the body and is divided by a terminal invagination of the body, giving the appearance of two very large testes, which after dissection showed two denser globular structures that were interpreted as the true testes; vitellaria extend in scattered compact clumps from level of intestinal bifurcation to terminal area, a collecting tubule running internally at ovary level; crura large, rising just anterior to the ventral sucker, a weak organ  $0.06$  mm. in diameter; oral sucker stronger but smaller,  $0.02 \times 0.016$  mm., bearing

spines; no prepharynx was noted; pharynx 0.01 mm. long; oesophagus present; seminal receptacle lateral and posterior to the ovary; seminal vesicle, a tubular structure, dorsal of ovary; gonotyl and genital pore lie just anterior to acetabulum.

#### FAMILY CLINOSTOMIDAE, Lühe, 1901

Larval specimens of the genus *Clinostomum* were taken from several hosts. They were encountered in the intestinal tracts of perch, rock and small-mouthed bass, and pike. None showed evidence of having reached the adult stage. They were also found as cysts in the gills, gill covers, mouth, and flesh of these fish. It is perhaps worthy of note in this connection that though the adult parasite is extremely common in the Great Blue Heron in this district, and the metacercaria is of frequent occurrence, no cercariae were obtainable from hundreds of *Helisomas* examined.

All the specimens belonged to the species *complanatum*, discussed by Van Cleave and Mueller (19) under the specific name *marginatum* Rudolphi 1819, following the classification of Price (15).

#### *Clinostomum complanatum*, Braun, 1814

HOSTS: *Ambloplites rupestris*

*Esox lucius*

*Micropterus dolomieu*

*Perca flavescens*

These linguiform flukes are very muscular. The only readily distinguishable features are the oral sucker set in its deep collar, the large strong acetabulum, and the two tandem testes in the posterior half of the body. These testes are roughly triangular with apices distal and are considerably lobed. The convoluted crura rise from a short oesophagus at the base of the oral cup and almost meet near the end of the body. Midway between the testes lies the small ovary. Traces of the developing uterine sac are to be seen running from the anterior testis toward the acetabulum and often coming in contact with it.

#### FAMILY STRIGEIDAE Railliet, 1919

##### Genus *Posthodiplostomum* Dubois, 1936

Encysted metacercaria belonging to this genus were found frequently during this survey. Rock bass and small-mouthed black bass almost invariably carry the parasite in the liver, and in addition they have been found in the swim bladder of these fish, as well as excysted in the intestine. In the latter case no typically adult structures were noted in any instance. All were assignable to the single species, *Posthodiplostomum minimum*.

#### *Posthodiplostomum minimum* (MacCallum, 1921) Dubois, 1936

HOSTS: *Micropterus dolomieu*

*Ambloplitis rupestris*

The body is divided typically into a fore- and hind-part. The strongly muscled fore-body bears the oral sucker, acetabulum, holdfast gland, and the

faintly discernable crura which rise close behind the oral sucker. A pharynx is sometimes traced. The rear-body contains the developing gonads. The metacercariae lie slightly curled in a generous ovoidal or globular cyst. Two specimens from the liver of a rock bass were at least twice as large as other specimens. The body differentiation was not as marked as is usual, but in neither case were there apparent characters of specific importance.

Formerly known as "*Neascus vancleavei*" (Agesborg) Hughes, this form has been shown by Ferguson (4) to be the metacercaria of *P. minimum*. Dubois (2) limits the generic designation *Neascus* to the larval stage of the sub-subfamily, Crassiphialini. In his monograph (3) he uses the name of the genus he established by dividing *Neodiplostomum* Railliet, to designate this form, *i.e.*, *Posthodiplostomulum*.

#### FAMILY AZYGIIDAE Odhner, 1911

Several forms taken from eels and one form from a pike were clearly members of the family, Azygiidae, as defined by Manter (10). There was great diversity of body form and structures, but intermediate similarities justify their consideration as a single genus and species.

#### *Azygia longa* Leidy, 1851

HOSTS: *Anguilla rostrata*

*Esox lucius*

The single specimen from the pike most closely resembles typical *longa* in superficial appearance. Body long and attenuate, suckers very prominent, oviduct extensive and conspicuous. All features typical: oral sucker  $0.68 \times 0.64$  mm.; body  $12.8 \times 0.64$  mm. with greatest width at level of acetabulum and just posterior to termination of vitellaria; spherical pharynx about half diameter of oral sucker, oesophagus short and deflected dorsally; crura extending to posterior margin of body; ventral sucker terminal to first quarter of body length and approximately equal in diameter to oral sucker; muscular and prominent genital pore only slightly anterior to this sucker and equal in diameter to or slightly less than the pharynx, the conspicuous genital sinus emptying through it; anterior vitellarian follicles 0.23 mm. behind the acetabulum to within 0.88 mm. of posterior end of body; testes medial and tandem separated by approximately their own diameter or less and 2.3 mm. from the posterior end.

Longer, wider specimens were taken from eels. Some of these could justifiably have been placed in the species *angusticauda*, following the description and figures of Van Cleave and Mueller (19), but no clear differentiation was possible from other forms that were obviously *longa*. Some measurements are given in Table I to indicate size and structure placement. The necessity for separation of these two species seems to be questionable.

TABLE I  
MEASUREMENTS OF SPECIMENS OF *Azygia longa*, IN MILLIMETRES

Specimen No.	Width at acetabulum, mm.	Length	Distance of acetabulum behind oral sucker	Vitellaria		Testes anterior to tip	Ovary anterior to testes
				Behind acetabulum	Anterior to tip		
1	4.8	15.12	1.36	0.16	2.0	5.3	0
7	1.6	12.4	1.6	0.08	2.72	3.68	0
4	1.12	12.8	1.04	0.4	2.0	5.6	0.16
9	1.6	11.2	1.28	0.16	1.12	3.2	0.61

#### FAMILY PTYCHOGONOMIDAE, Dolfuss, 1937

Three specimens taken from the stomachs of three otherwise parasite-free yellow perch from Lake Commandant were not referable to any family previously recorded from Percidae. They were adult and egg-bearing, and as the perch, after capture, had been kept in a controlled tank for several weeks prior to killing, we may conclude that these fish were suitable hosts, in spite of the small infection.

The flukes were heavily muscled and filled with large eggs. Because of this, examination was difficult, but they are identifiable as members of the genus *Ptychogonimus*. Previously this genus has not been represented in freshwater collections and has been limited to a single species *megastomum*, found most frequently in sharks. However, the closely related family *Azygiidae*, from which this family has been separated because it has developed a functional anus (1), is known to occur in both freshwater and ocean fish (10).

Apart from the host and habitat differences, the present form varies in having oblique testes, while *P. megastomum* has tandem testes. Though otherwise very like the salt-water form, it is justifiable to consider this as representing a new species. The designation *Ptychogonimus fontanus* sp. nov. is proposed for it.

#### *Ptychogonimus fontanus* sp. nov.

Host: *Perca flavescens*

The specimens are markedly contracted, being only slightly over 1.0 – 1.5 mm. long and about 0.5 mm. in width. Oral sucker subterminal, strongly muscled, about 0.25 mm. in maximum diameter; prepharynx short, not extending beyond caudal-edged sucker; pharynx goblet-shaped, large and strong, about 0.14 mm. long; no oesophagus; intestinal crura leave pharynx directly, pass anteriorly to oral sucker, then turn and run posteriorly to the terminal area of the body, where they are in contact with the excretory bladder and probably open into it. Genital opening within large sucker-like sinus in line with and slightly left of pharynx, anterior to acetabulum, provided with ejaculatory duct and seminal vesicle. Acetabulum large, 0.196 × 0.235 mm.,

strong concentric muscles. Oral sucker, pharynx, genital sinus, and acetabulum closely crowded together. Ovary and most of testes obscured by egg-filled uterus, which reaches posteriorly midway down testes and passes left and behind acetabulum to genital pore; two testes, strongly oblique, close together near end of body; entire, transversely ovoid; ovary entire, only slightly anterior to testes; intra-uterine eggs numerous, yellow, varying in size and shape but typically a broad oval about  $0.0056 \times 0.0035$  mm. Vitellarian follicles separate, pyriform or oval, lateral from midline of acetabulum to posterior margin of posterior testis, extending dorsally toward midline at this point and around common vitelline duct at level of ovary. Excretory sac intercrural at posterior limit, communicating with exterior by tube through musculature and in close association with crura. Longitudinal muscle bands complete, distinct; transverse muscles not distinct. The cuticle is provided throughout with many granulosities without apparent arrangement.

### Host Parasite List

<i>Acipenser fulvescens</i>	<i>Esox masquinongy</i>
<i>Crepidostomum lintoni</i>	<i>Phyllodistomum staffordi</i>
<i>Ambloplites rupestris</i>	<i>Ictalurus punctatus</i>
<i>Crepidostomum cornutum</i>	<i>Crepidostomum ictaluri</i>
<i>Centrovarium lobotes</i>	<i>Vietosoma parvum</i>
<i>Clinostomum complanatum</i>	
<i>Posthodiplostomum minimum</i>	<i>Micropterus dolomieu</i>
<i>Ameiurus nebulosus</i>	<i>Clinostomum complanatum</i>
<i>Acetodextra amiura</i>	<i>Crepidostomum cornutum</i>
<i>Centrovarium lobotes</i>	<i>Posthodiplostomum minimum</i>
<i>Anguilla rostrata</i>	<i>Perca flavescens</i>
<i>Azygia longa</i>	<i>Crepidostomum cooperi</i>
<i>Crepidostomum brevivitellum</i>	<i>Bunodera sacculata</i>
	<i>Clinostomum complanatum</i>
<i>Esox lucius</i>	<i>Ptychogonimus fontanus</i>
<i>Azygia longa</i>	
<i>Centrovarium lobotes</i>	<i>Stizostedion vitreum</i>
<i>Phyllodistomum superbum</i>	<i>Centrovarium lobotes</i>
<i>Clinostomum complanatum</i>	<i>Bucephalopsis pusilla</i>

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## AN APPARATUS FOR MEASURING THE "FLASH" THERMAL DEATH POINT OF MICROSCOPIC ANIMAL ORGANISMS AND ITS USE WITH OVA OF *ASCARIS LUMBRICOIDES*<sup>1</sup>

BY W. E. SWALES<sup>2</sup> AND DAROL K. FROMAN<sup>3</sup>

### Abstract

A method of measuring "flash" thermal death points of microscopic animal organisms is described. By means of the devised apparatus the time of exposure can be varied from 0.5 to 0.1 second, and the temperature can be estimated with relative accuracy. In a sample determination, the single-celled ova of *Ascaris lumbricoides* (porcine origin) were all destroyed at a temperature of 68° C. in an exposure of 0.44 second.

The free-living stages of certain animal parasites have been shown by numerous workers to be extremely resistant to the action of chemical substances. It has been shown, however, that the destruction of such organisms can usually be accomplished by thermal means. Hot water is commonly used in applied parasitology to destroy the eggs of nematodes and the cystic stages of certain protozoa parasitic in man and higher animals, particularly when these organisms are present in buildings and enclosures as potential sources of infection. Water and aqueous solutions of chemicals at high temperatures have been proved to be highly efficacious as ovicides. In agricultural parasitology such agents are highly practical in prophylactic measures against nematodes of the families Ascaridae, Heterakidae, Oxyuridae and Trichinellidae, and against the many species of coccidia that infest mammals and birds. The McLean County system of sanitation for the prevention of roundworm and other infections of pigs relies upon the destruction of the ova in breeding pens by the use of hot water.

In devising practical methods of destroying infection in breeding pens for pigs in eastern Canada, it became evident that exact knowledge regarding the efficacy of methods was not readily available. Roberts (5) had tested the resistance of ova of *Ascaris lumbricoides* against hot water at temperatures ranging from 50 to 100° C., and had found that they were destroyed at 70° C. in from one to two seconds, and at 80, 90, and 100° C. in a fraction

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of a second. Wharton (6) had shown that all eggs of *A. lumbricoides* were killed at 70° C., and Ogata (3) had shown that they were destroyed in one second at 70° C., in more than 50 sec. at 65 ° C., and in more than 45 min. at 50° C.

It seemed highly desirable, for practical purposes, to set a tentative "flash" thermal death point for the ova of *Ascaris* and for similar organisms, so that standard methods of sanitation would have a definite basis. Consideration of factors that would influence the contact of water with the egg under practical conditions indicated that the time factor would have to be very short, particularly when the work was carried out in cold weather. Thus it was considered that some point below half a second would serve as a practical time standard for the measurements of thermal death points of free-living stages of animal parasites. A description of the technical methods employed by Ogata was not available. Roberts had made his measurements by drying the ova on glass slides and exposing them to hot water by rapid immersion; it was apparent that this method would involve protection of the eggs to some extent through the heat capacity of the glass slide, particularly in very short exposures, and thus was not considered suitable as a standard method.

In our investigation, an apparatus has been devised that appears to eliminate errors to a large extent.

### Description of Apparatus and Methods

The problem of maintaining a microscopic egg or oöcyst at any desired temperature for a short, but measurable, time was solved by projecting an excess of the organisms into a vertical stream of heated water; the stream carried the organisms into a large volume of water. It was assumed that the time required by an individual organism to attain the temperature of the stream was equal to the time of cooling in the cold water bath, and that this time factor was negligible; thus the time of exposure was taken to be the time the organism remained in the stream. This assumption is supported by the example that if the surface of an egg of *Ascaris lumbricoides* at room temperature be suddenly raised to 60° C. and maintained at that temperature, an approximate calculation shows that the centre of the egg will be above 59.9° C. within 1/1000 sec.

Let  $h_1$  be the distance the water has fallen under the action of gravity to the point at which the organism is introduced into the stream. Let  $h_2$  be the total height through which the water falls, the distance  $h_2$  being measured from the surface of the water in a releasing cup, shown as  $F$  in Fig. 1, to the

FIG. 1. A diagram of the apparatus as arranged in a test.

A. Vessel for hot water, equipped with knife heater and thermometer. B. Vessel for suspension of organisms. C<sub>1</sub>. Inner glass tube. C<sub>2</sub>. Outer glass tube, forming hot water jacket. D. Vessel of water for cooling organisms. E. The apparatus for selecting central stream (S) and discarding splashed or delayed organisms (shown enlarged in Fig. 1c). F. Release cup for forming vertical stream (S). G. Galvanometer. H. Finely drawn capillary tube for injecting suspension of organisms. J<sub>1</sub> and J<sub>2</sub>. Thermocouple junctions. K. Tube conveying hot water. P<sub>1</sub> and P<sub>2</sub>. Motor-driven stirring paddles. R. Variable resistance. S. Vertical stream. T. Sealed glass tube containing thermocouple wires. FIG. 1b. Enlarged diagram of thermocouple junction (J<sub>1</sub>).

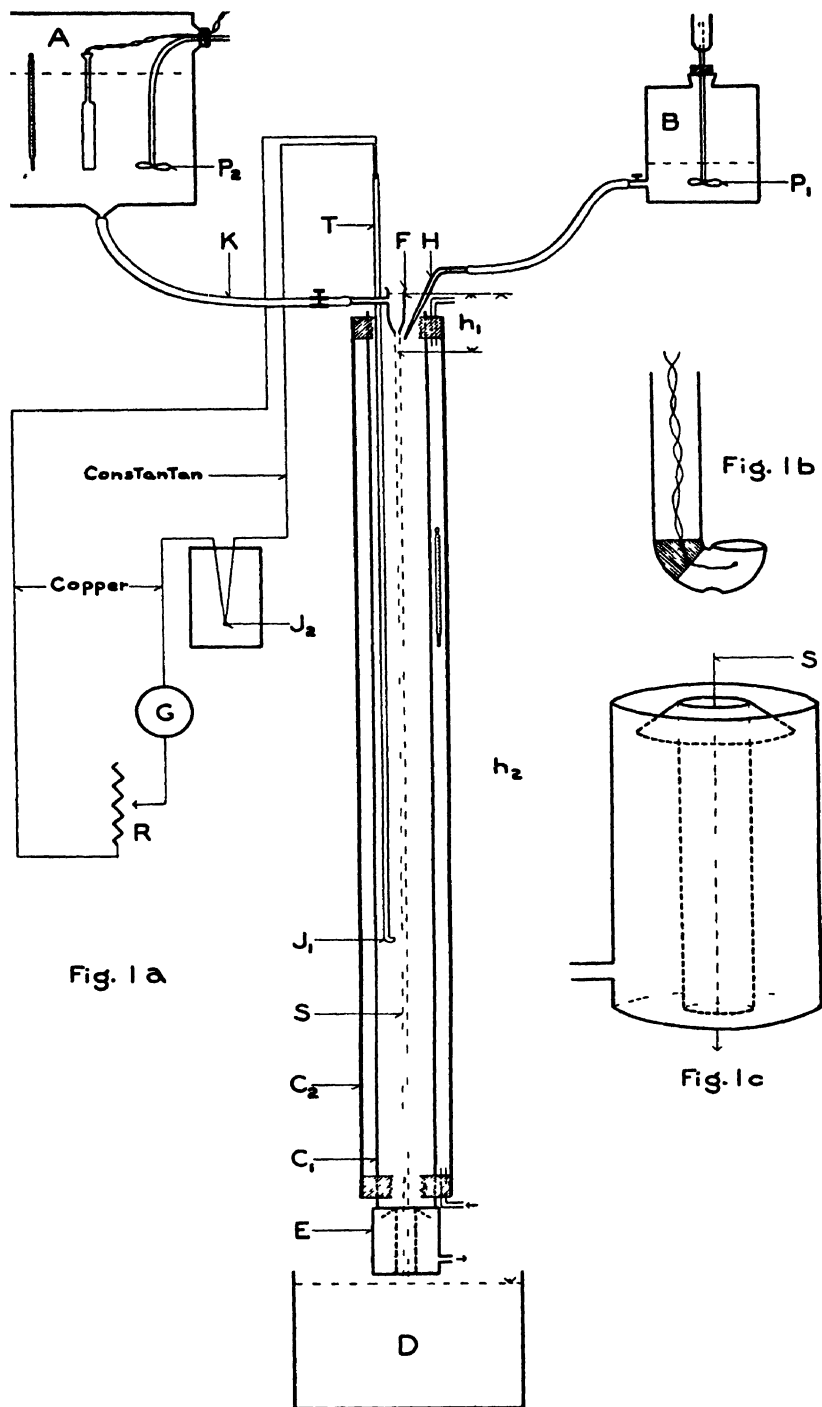


FIG. 1

surface of the cold water. Then the velocity ( $v_1$ ) of the stream at the point of entrance of the organisms is given by

$$v_1 = \sqrt{2gh_1}$$

where  $g$  is the acceleration of gravity. The velocity of the stream at the surface of the cold water is

$$v_2 = \sqrt{2gh_2},$$

hence the time of exposure is

$$t = \frac{2(h_2 - h_1)}{v_1 + v_2} = \sqrt{\frac{2}{g}} (\sqrt{h_2} - \sqrt{h_1}). \quad (1)$$

Experience shows that  $h_1 = 7$  cm. is a convenient distance. Taking  $h_1 = 7$  cm. and  $g = 981$  cm./sec.<sup>2</sup>, Table I has been calculated from Equation (1) for certain values of  $h_2$ .

TABLE I  
THE TIME ( $t$ ) REQUIRED FOR AN OBJECT TO FALL UNDER GRAVITY FROM THE POINT OF ENTRANCE OF THE ORGANISMS INTO STREAM ( $S$ ) TO COLD WATER SURFACE

$t$ , sec.	$h_1$ , cm.	$h_2$ , cm.
0.1	7.0	21.6
0.2	7.0	50.0
0.3	7.0	86.2
0.4	7.0	132.3
0.5	7.0	188.2

The apparatus was arranged as shown in Fig. 1. Water was heated to any desired temperature by means of the immersed "knife-type" heater in a 6-litre vessel,  $A$ , and was stirred by a motor-driven paddle (flexible shaft). The water was allowed to flow through a tube,  $K$ , at a speed required to maintain a constant level in a cup,  $F$ . The water fell under gravity from an aperture in the bottom of the cup into an 8-litre container,  $D$ , nearly filled with cold water. The organisms under study were kept at room temperature in a 1-litre bottle,  $B$ . A small stream of water carrying these organisms was projected into the main stream,  $S$ , from a finely drawn glass tube,  $H$ , at a measured depth,  $h_1$ , below the water level in the cup. The water at this point was falling with the speed a freely falling body would attain in falling a distance  $h_1$  (Torricelli's theorem). The time of exposure to the high temperature was calculated by means of Equation (1) from the observed values of  $h_1$  and  $h_2$  (Fig. 1).

In order to keep the temperature of the stream constant during the fall, it was surrounded by two glass tubes,  $C_1$  and  $C_2$ . Hot water was circulated between these tubes. The inner tube was 4.4 cm. outside diameter and about 140 cm. long, with a 2-mm. wall. The outer tube was 8 cm. outside

diameter and about 125 cm. long, with a 2-mm. wall. It was found that practically no change in temperature occurred along the falling stream if the jacket temperature was kept within 10° C. of the stream temperature.

Owing to a small amount of splashing, some water collected on the inside of the tube  $C_1$  and ran down the walls. This water was discarded by means of the collecting can,  $E$ . The flange on the top of the inner cylinder of this can prevented any splashed water from falling into the vessel,  $D$ , and, in fact, only the central stream was admitted into vessel  $D$ .

The temperature of the water at any desired point in the stream was measured by means of copper-constantan thermocouple junction,  $J_1$ , projecting from the end of a glass tube,  $T$ . This junction is shown enlarged in Fig. 1b. The ends of the wires were painted with "Glyptal" after being soldered, and the end of the glass tube was shaped so that the junction was continuously surrounded by the water in the stream. The small hole in the bottom of the catch-cup allowed free circulation of the water. The temperature of the stream was measured at three definite levels immediately before and after each test. The thermocouple was kept completely out of the stream during a run while the container  $D$  was in place. Thus no organisms which might have been held momentarily on the end of the tube  $T$  could get into  $D$ . The second junction,  $J_2$ , was kept at 0° C. in a mixture of ice and water contained in a Dewar (or Thermos) flask. The temperature was read by means of a dead-beat galvanometer,  $G$ . (Any linear galvanometer with a sensitivity of about 0.02 microamps per division is suitable.) The temperature sensitivity was about 2.5 divisions per degree C. The galvanometer scale was calibrated by immersing junction  $J_1$  in a beaker of water, whose temperature was taken with a standard mercury thermometer. This calibration was checked frequently, and any necessary small corrections to the galvanometer readings were made by adjusting the resistance,  $R$ . The resistance used was a 0 to 10,000 ohm box, variable in steps of one ohm. (A 1000 ohm rheostat would be suitable.) An alternative method of reading the temperature would be the use of a potentiometer and null-point galvanometer.

Rough estimates of the probable errors in the temperature measurements were made from the variations of temperature along the stream during the time of a run, as well as from the precision of the thermocouple system. The temperature can be measured and maintained constant within a probable error of about 0.1° C.

### Use of the Apparatus. Tests on Single-celled Eggs of *Ascaris lumbricoides* (Porcine Origin)

For the purpose of demonstrating the apparatus, eggs of *A. lumbricoides* were used. Mature worms were collected in abattoirs as they were removed from the intestines of newly killed pigs and were promptly immersed in physiological saline at 38 to 40° C. In the laboratory they were cleaned and

placed in fresh saline in an incubator at body temperature. The eggs produced by these worms, during the first 24 hr. only, were used, after being washed and stored in water at 4° C. On each day on which tests were made the eggs were allowed to slowly regain room temperature, and large numbers were then suspended in container *B*. The suspension was kept in continual motion by means of the motor-driven stirring paddle, *P*<sub>1</sub>.

In these tests the original apparatus was used, *t* having been found to have the value of 0.44 sec. after measurements of *h*<sub>2</sub> had been made. As the original object was to test the effect of exposures to certain temperatures for a period of less than  $\frac{1}{2}$  sec., this point of 44/100 sec. was considered as a satisfactory "flash point" for preliminary tests.

The apparatus was further prepared by almost filling vessel *A* with water and heating it to a temperature slightly above the point it was required to test; the top of the container was sealed for the duration of each test. Trials of the vertical stream and the mixture of stream and suspension of eggs were made in order to ensure that the centre of the stream would fall through the aperture in the cup *E*. The space between tubes *C*<sub>1</sub> and *C*<sub>2</sub> was then filled with hot water (at a temperature within 10° C. of that of the proposed test), which continued to flow by tap pressure at a controlled rate throughout each test. The vessel of cold water, *D*, (16 to 18° C.) was placed near the apparatus, and the main vertical stream and the small stream of egg suspension were allowed to fall into a temporary container in place of vessel *D*. Temperatures of the stream were then read in units on the galvanometer scale by one operator, as the other operator placed the thermocouple junction, *J*<sub>1</sub>, in the vertical stream at three points. If the temperature was satisfactory and if the three points did not vary more than 0.1° C., a test was immediately taken by placing vessel *D* at the base of the stream for a period of about 30 sec., during which time one operator stirred the cold water in vessel *D*, and the other ascertained that the apparatus was working correctly. At the end of this period, vessel *D* was quickly replaced by the temporary vessel. The temperatures of the stream at the same three points were again taken, and the operation of the apparatus was stopped. If the subsequent examination of the six records of temperature showed that the stream had remained at a constant temperature or that the highest did not vary from the lowest reading by more than 0.2° C., the test was recorded as satisfactory. By a simple process of sedimentation, the eggs that had fallen into vessel *D* were recovered, and several thousands were placed in "Syracuse" watch glasses, where they were distributed evenly over the bottom and allowed to dry. A similar number was recovered from the suspension in vessel *B*, and these unexposed eggs were dried on another watch glass for control observations. As soon as the eggs had dried, each set was labelled, placed in a moist chamber, and kept at 27° C.

The criterion of survival adopted was the ability of each egg to form an active embryo. After two weeks, 500 eggs were examined microscopically as a preliminary estimation of results. In examinations made two months

after each test, it was found that in some preparations, which showed no survival at the first reading, a few eggs would have recovered enough to form living embryos. Therefore, in the data given in Table II, the results of examining 500 eggs two months after each test are used as the final figures of percentage survival.

Seventeen satisfactory tests have been made to determine the "flash" thermal death point of single-celled ova of *A. lumbricoides*, the results being shown in Table II and Fig. 2.

In Fig. 2 the diameters of the circles around the plotted points show, approximately, the standard errors in the temperature determinations. The lengths of the vertical lines represent the expected standard errors due to sampling (500 eggs) in estimating the percentage survival.

TABLE II  
THE EFFECT OF EXPOSURE OF SINGLE-CELLED OVA OF *A. lumbricoides* TO HOT WATER  
FOR 44/100 SECOND

	Temp., °C.	Exposed eggs, % living embryos	Control, % living embryos	Per cent survival
1	56.0	99.0	99.0	100.0
2	60.5	99.2	99.0	100.0
3	61.5	99.0	99.0	100.0
4	63.0	97.7	98.2	99.5
5	63.75	98.7	98.8	100.0
6	64.0	93.9	98.2	95.6
7	64.5	93.5	98.8	94.6
8	64.5	88.8	98.0	90.6
9	65.4	90.4	98.8	91.5
10	65.5	55.1	98.0	56.2
11	65.6	52.6	98.8	53.2
12	66.2	13.9	98.2	14.2
13	66.2	5.6	99.0	5.7
14	66.4	30.5	98.8	30.9
15	66.8	7.1	98.8	7.2
16	67.4	2.6	98.2	2.6
17	68.0	0.0	99.0	0.0

### Discussion

The results of these determinations form a surprisingly steep curve. The critical death point of single-celled eggs of *Ascaris lumbricoides* is, apparently, between 67 and 68° C., although the majority are destroyed at points between 66 and 67° C., when exposed for a period of 0.44 sec. These results are not in complete accord with those of previous workers, in that they show that all the eggs are destroyed in 0.44 sec. at a lower temperature than those reported. We believe that the difference may be due to the fact that our method eliminates such factors as the heat capacity of the object used to hold the eggs during exposure in previous methods, and thus ensures that the egg reaches and is maintained at the temperature for a known "flash" period. As the

results became evident, every effort was made to detect possible mechanical errors, but none was found; and the method has now been described as a practical means of determining "flash" thermal death points for similar animal organisms, and possibly for plant organisms.

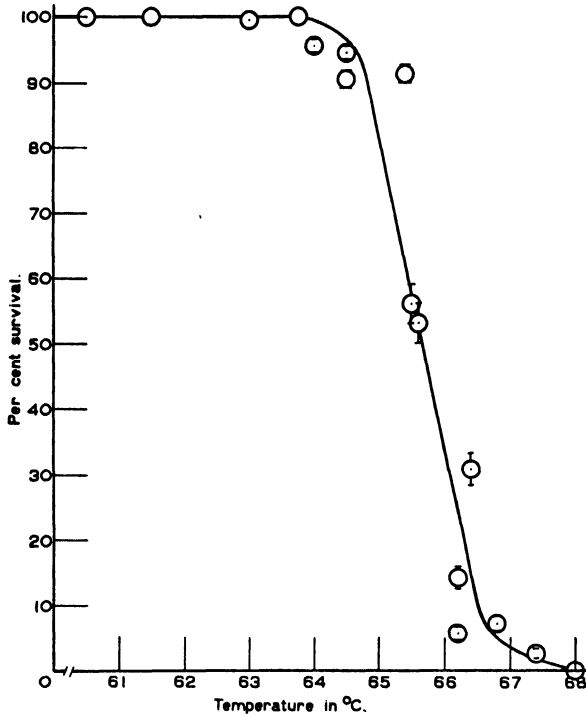


FIG. 2. The resistance of single-celled ova of *Ascaris lumbricoides* to exposure in hot water for 0.44 sec. Circles and vertical lines represent standard errors.

The time factors used in such measurements of "flash" thermal death points cannot replace the standard time of 10 minutes used by biologists in estimating the thermal death point of organisms, but accurate estimations of the effects of exposures of less than half a second would have direct application in designing measures of control against pathogenic organisms. For example, "flash-point" measurements would have value in extending such tables as those published by Reinhardt and Becker (4) on the effect of temperature on infectivity of coccidial oöcysts.

It is felt that these preliminary results justify publication of the general description of the apparatus and methods. In building a similar apparatus, other workers will no doubt make modifications according to their facilities, and for this reason no description has been given of minor details. Some difficulties may be experienced with the vertical stream, but adjustment of the form of the outlet will overcome excess splashing.

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## FOOD OF DUCKS AND COOTS AT SWAN LAKE, BRITISH COLUMBIA<sup>1</sup>

BY J. A. MUNRO<sup>2</sup>

### Abstract

A study of the autumn food habits of ducks and coots at Swan lake, British Columbia, based on the examination of 136 stomachs and on co-related field work, indicates the following conclusions: pond ducks had eaten 78% plant material, 12% *Chara*, and 10% animal organisms; the food of diving ducks was 65% plant material, 31% *Chara*, and 15% animal matter; while that of coots was 97% *Chara* and 3% plant material. *Chara* is the dominant growth in the lake. It is produced in unlimited quantities so that the food requirements of coots do not seriously compete with those of ducks.

### Introduction

In order to obtain precise information on the food relations between coots and ducks as it applied to one particular area, a study of bird populations and their food was undertaken at Swan lake, near Vernon, British Columbia. This body of water is typical of certain marshy lakes which are known to be the common nesting grounds for various species of ducks and for the American Coot, *Fulica americana americana*, as well as being seasonal concentration places for the coot. In the autumn, when the local coot population has been increased many times by an influx of migrants, the total number is impressive. The coots associate in large flocks on open water, where they are more conspicuous than are the scattered diving ducks and Baldpate, *Mareca americana*, which usually accompany them, while the population of pond ducks, which frequent inshore shallows and sheltering marsh growth, may escape observation altogether. Thus the disproportion in numbers of ducks and coots may appear greater than it actually is, although the autumn concentration of coots may greatly outnumber the total of all species of ducks present.

The investigation was carried on at intervals during the years 1932 to 1937 inclusive and comprised population counts of waterfowl at different seasons, the collection and identification of plants and animal food organisms, and the collection for the purpose of stomach analyses of ducks and coots during the autumn months, when greatest concentration occurred. As the study has been a local and seasonal one, the conclusions reached regarding the food relations are not necessarily applicable to spring and summer periods, nor to lakes in which different physical, chemical, and biological conditions exist.

### Numbers of Waterfowl

The figures given in enumerations of waterfowl as set forth in Table I represent exact counts in some instances; in others it was necessary to rely upon estimates made as carefully as conditions would permit. It is believed

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Contribution from the National Parks Bureau, Department of Mines and Resources, British Columbia, Canada.

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TABLE I  
ENUMERATIONS OF DUCKS AND COOTS, SWAN LAKE, BRITISH COLUMBIA

	Common Mallard	Gadwall	Baldpate	American Pintail	Green-winged Teal	Blue-winged Teal	Cinnamon Teal	Shoveller	Redhead	Ring-necked Duck	Canvas-back	Scaup Ducks†	American Golden-eye	Barrow's Golden-eye	Buffle-head	White-winged Scoter	Ruddy Duck	Total of ducks	American Coot
May 16, 1932	20	-	2	2	-	14	20	4	200	-	2	4	2	3	-	10	40	323	1000
Sept. 15	200	50	20	100	300	200	*	3	400	-	-	-	-	20	30	5	500	1828	800
Sept. 20	100	6	6	100	250	10	-	1	50	-	-	7	-	6	30	-	200	766	765
Nov. 11	-	-	-	-	-	-	-	-	30	-	-	400	50	-	100	-	-	580	700
May 11, 1933	10	2	-	-	4	13	7	-	118	-	5	8	-	17	57	-	67	295	150
Sept. 30	300	5	50	400	400	50	-	-	10	-	15	20	10	1	10	1	8	-	2500
Nov. 1	100	4	15	200	75	2	-	-	60	12	200	60	10	-	100	50	889	6000	
Nov. 4	200	-	150	250	100	-	-	75	50	10	50	300	10	-	200	1	1395	6000	
17	1000	-	30	40	-	-	-	8	1500	5	100	500	10	-	30	5	3223	4000	
Sept. 29, 1934	350	50	300	150	250	100	-	75	1	5	-	15	-	-	-	-	30	1311	3000
Oct. 4	100	30	500	300	275	6	-	10	-	-	20	15	-	-	8	-	1	488	2000
Oct. 18	100	50	50	50	50	20	10	10	70	7	150	-	-	-	6	-	150	399	750
Sept. 15, 1935	90	-	10	-	20	30	-	10	25	12	1	-	-	12	-	10	30	243	1500
Sept. 23	75	10	30	-	10	30	-	10	119	3	-	6	-	3	20	-	104	277	644
May 2, 1936	3	3	-	-	-	-	11	5	222	5	-	20	-	3	14	-	22	312	81
May 20	14	-	1	-	-	6	4	4	6	222	-	6	1	-	-	-	3	245	61
June 4	3	2	-	-	-	2	8	4	211	5	-	6	-	3	1	-	161	941	1500
Sept. 12	202	5	35	-	101	133	-	1	299	-	-	-	-	-	-	-	60	439	1000
Sept. 21	130	35	30	6	80	28	-	-	70	-	-	-	-	1	-	-	20	320	108
June 12, 1937	32	3	-	-	16	14	-	6	214	-	-	14	-	1	-	-	20	1850	2500
Oct. 1	250	10	400	140	800	140	-	30	40	5	4	6	-	-	5	-	12	434	2500
Oct. 8	38	14	250	10	50	28	-	6	15	-	1	5	-	6	-	-	20	252	86
June 4, 1938	28	13	13	-	-	8	6	20	143	-	1	-	-	-	-	-	20	252	86

Not recorded above—Old-squaw; Sept. 30, 1933, 1; Nov. 1, 1933, 2.  
 Hooded Merganser: April 4, 1932, 3; Nov. 1, 1933, 3.  
 American Merganser: May 26, 1932, 4; May 2, 1936, 11.  
 \* Autumn counts of Blue-winged and Cinnamon teal are combined.  
 † Scaup ducks are not recorded specifically.

that a fair degree of accuracy in estimating waterfowl populations can be achieved through constant practice and that the margin of error is constant enough so that the figures have comparative value.

The following species and numbers of birds were used in the stomach examinations:—

Common Mallard— <i>Anas platyrhynchos platyrhynchos</i>	12	Redhead— <i>Nyroca americana</i>	8
Gadwall— <i>Chaulelasmus streperus</i>	5	Ring-necked Duck— <i>Nyroca collaris</i>	5
Baldpate— <i>Mareca americana</i>	9	Canvas-back— <i>Nyroca valisineria</i>	1
American Pintail— <i>Dafila acuta tzitzihua</i>	10	Greater Scaup Duck— <i>Nyroca marila</i>	1
Green-winged Teal— <i>Nettion carolinense</i>	10	Buffle-head— <i>Charitonetta albeola</i>	6
Blue-winged Teal— <i>Querquedula discors</i>	2	Old-squaw— <i>Clangula hyemalis</i>	2
Cinnamon Teal— <i>Querquedula cyanoptera</i>	5	Ruddy Duck— <i>Erismatura jamaicensis rubida</i>	9
Shoveller— <i>Spatula clypeata</i>	6	American Coot— <i>Fulica americana americana</i>	45

These were collected during the months of September, October, and November in the years 1933 to 1937. As no appreciable difference was apparent in the food taken in different years it has not been considered necessary to treat year groups separately.

Study of the stomach material was made at the Pacific Biological Station, Nanaimo, British Columbia, and the identification of aquatic insects was largely the work of the Director, Dr. W. A. Clemens. For this co-operation and for the use of the laboratory facilities of the Biological Station, the author wishes to make grateful acknowledgment.

### Description of Lake

Swan lake, slightly over three miles long with a maximum width of about 1,000 yards, is situated in a narrow valley of farm lands which, in orchard and pasture, extend up the open hills to the east and west. The maximum depth is 24 ft. (June 24, 1922); the bottom is partly humus on clay and partly marl, with smaller areas of sand and gravel. B. X. creek, which enters the south end of the lake, is the chief tributary, and the spring flood water from this source causes a rise of water level which reaches the peak in early June. This supply usually fails before midsummer as a result of water diversion for irrigation; subsequently the water level rapidly falls through evaporation, so that portions of the lake bottom in the shallows may be

exposed by September. At this time also, sheltered portions of the lake are usually covered with the "bloom" of blue-green algae, which disappears with the onset of colder weather in the autumn. The shores, except for a few stretches of open, muddy beach, are encircled with a belt of bulrushes and cat-tails which widens into marshes at either end. On the east and west shores, between high water mark and the edge of cultivation, is a discontinuous growth of alder, willow, black haw, mountain birch, and several small stands of trembling aspen.

### Analyses of Stomach Contents

The results of the examination of the contents of waterfowl stomachs, shown in Tables II to IV, are here briefly summarized.

The food of 59 pond ducks of eight species collected during the months of September, October, and November comprised 78% vegetable matter, 12% *Chara* branches and oospores and 10% animal organisms, chiefly molluscs and insects. Seeds of aquatic plants, with *Scirpus* first and *Potamogeton* second, were the most important both in respect to time of occurrence and average percentage volume.

The food of 32 diving ducks taken at the same place during the same period consisted of 54% plant material with *Scirpus* seeds predominating, 31% *Chara* branches and oospores and 15% animal matter.

Forty-five coots taken at the same place during the same period had eaten *Chara* branches almost exclusively, the total percentage volume, including two small items of oospores, being 97%; the remainder consisted of other plant materials. The high percentage of *Chara* is taken to indicate a decided food preference; *Chara* oospores apparently are not sought for as would seem to be the case with ducks. *Scirpus* seeds, which occurred in small numbers in 25 stomachs, were in each case a minor item and probably taken incidentally to the *Chara*.

### Cover and Food Plants

Bulrush—*Scirpus occidentalis*. Of the emergent plants the bulrush is the most important in the economy of the lake, providing cover and nesting material for many kinds of birds, as well as furnishing a seed crop of the highest value. Dry plants of the previous year's growth are used as nesting material by Redhead, Canvas-back, Ruddy duck, and coots; the thick clumps of dry growth, that have resisted winter storms and the weight of heavy snow, provide nesting cover; the rotted stems are often the chief constituent in the floating nests of grebe; the green stems represent the chief item in the diet of muskrats and are used in building their houses; many kinds of aquatic insect larvae use the stems as emerging ladders; molluscs cling to the under-surface portions of the plants; and finally the thick growth provides shelter for the propagation of lesser water plants and numerous small aquatic organisms.

The seeds are eaten by ducks, and to a lesser extent by coots, rails, waders, and other birds. The seeds ripen and begin to fall in September; many

TABLE II  
AUTUMN FOOD OF DUCKS AND COOTS, SWAN LAKE, BRITISH COLUMBIA. NUMBER OF OCCURRENCES OF FOOD ITEMS

	Number of specimens	<i>Chara</i> branches	<i>Chara</i> oospores	<i>Scirpus</i> seeds	<i>Potamogeton</i> seeds	Other seeds	Molluscs	Insects	Miscellaneous animals	Miscellaneous vegetation
Mallard	12	-	-	11	10	5	5	1	-	10
Gadwall	5	-	1	5	-	-	-	1	1	2
Baldpate	9	2	2	2	-	-	-	1	1	8
American Pintail	10	2	6	10	7	5	-	-	-	-
Green-winged Teal	10	-	7	9	3	2	1	4	-	2
Blue-winged Teal	2	-	1	1	-	1	2	1	-	-
Cinnamon Teal	5	-	1	5	5	2	4	5	-	2
Shoveller	6	-	2	4	5	2	6	-	1	1
Redhead	8	3	4	5	4	1	1	1	1	5
Ring-necked Duck	5	1	4	5	2	1	1	-	-	1
Canvas-back	1	1	-	5	1	1	-	-	-	-
Greater Scaup Duck	1	-	-	-	1	-	-	-	-	-
Buffle-head	6	-	-	6	5	-	2	4	4	2
Oldsquaw	2	2	-	2	1	2	-	-	-	-
Ruddy Duck	9	-	5	8	1	1	-	1	1	2
American Coot	45	45	2	31	1	3	-	-	-	3

TABLE III  
AUTUMN FOOD OF DUCKS AND COOTS, SWAN LAKE, BRITISH COLUMBIA, AVERAGE PERCENTAGE VOLUME

	Number of specimens	<i>Chara</i> branches	<i>Chara</i> oospores	<i>Scirpus</i> seeds	<i>Pota- mogon</i> seeds	Other seeds	Molluscs	Insects	Miscel- laneous animals	Miscel- laneous vegetation
Mallard	12	—	—	56.84	13.00	2.75	2.00	0.08	—	25.33
Gadwall	5	—	0.40	70.60	—	—	—	—	9.60	19.40
Baldpate	9	21.44	0.44	0.22	—	—	—	0.11	0.11	77.68
American Pintail	10	6.20	24.40	63.50	2.30	3.60	—	—	—	—
Green-winged Teal	10	—	24.30	39.80	5.70	6.00	0.60	10.10	—	13.50
Blue-winged Teal	2	—	0.50	45.00	—	2.50	14.50	37.50	—	—
Cinnamon Teal	5	—	—	65.20	4.80	0.60	25.20	3.40	—	0.80
Shoveller	6	—	0.50	24.83	50.00	0.83	23.00	—	0.17	0.67
Summary, pond ducks	59	4.32	8.40	44.82	9.48	2.38	5.45	3.28	0.85	21.02
Redhead	8	31.25	16.63	20.87	11.00	1.13	1.00	0.12	1.13	16.87
Ring-necked Duck	5	1.00	34.00	56.20	2.20	0.40	0.20	—	1.00	5.00
Canvas-back	1	50.00	—	—	40.00	10.00	—	—	—	—
Greater Scaup Duck	1	100.00	—	—	—	—	15.14	—	—	—
Buffle-head	6	—	—	25.60	19.13	—	—	13.13	22.50	4.50
Old-squaw	2	72.50	—	6.50	0.50	20.50	—	—	—	—
Ruddy Duck	9	—	16.70	52.67	—	0.22	—	17.75	0.11	12.55
Summary, diving ducks	32	17.15	14.15	34.28	7.92	1.96	3.11	7.48	4.65	9.30
American Coot	45	96.60	0.13	2.87	—	0.06	—	—	—	0.34

TABLE IV

FREQUENCY OF OCCURRENCE OF IDENTIFIED FOOD SUBSTANCES IN STOMACH CONTENTS OF  
59 POND DUCKS, 32 DIVING DUCKS, AND 45 COOTS

Item	Pond ducks	Diving ducks	Coots
Musk-grass, <i>Chara</i> sp.	5	8	45
<i>Chara</i> oospores	21	13	2
Bulrush, <i>Scirpus occidentalis</i> , seeds	46	26	—
sp.	—	—	31
Pondweeds, <i>Potamogeton heterophyllus</i> , seeds	11	10	1
<i>foliosus</i> , seeds	2	1	—
<i>pectinatus</i> , seeds	26	6	—
<i>pectinatus</i> ; rootlets	1	—	—
Horned pondweed, <i>Zannichellia palustris</i> , leaves	2	—	1
Knotweed, <i>Polygonum amphibium</i> , seeds	—	—	1
<i>aviculare</i> , seeds	3	—	—
<i>muhlenbergii</i> , seeds	2	—	—
Smartweed, <i>Polygonum hydropiper</i> , seeds	1	—	—
Black bindweed, <i>Polygonum convolvulus</i> , seeds	1	—	—
Filamentous algae, <i>Spirogyra</i> , <i>Zygnema</i>	3	2	—
Sedge, <i>Carex</i> sp., seeds	2	1	—
Grasses, Gramineae, leaves	5	—	—
<i>Bromus</i> sp., leaves	—	—	1
Water milfoil, <i>Myriophyllum spicatum</i> , seeds	6	2	—
Golden dock, <i>Rumex maritimus</i> , seeds	2	1	—
Hornwort, <i>Ceratophyllum demersum</i> , seeds	1	3	—
Bur-reed, <i>Sparganium</i> sp., seeds	—	1	—
Cat-tail, <i>Typha latifolia</i> , seeds	1	—	—
Nostoc, cells	1	—	—
Comminuted vegetable matter	10	6	—
Freshwater sponge, Porifera	1	1	—
Bristleworm, <i>Nais</i> sp.	1	—	—
Water boatmen, Corixidae	6	6	—
Midge, Chironomidae, adults	2	—	—
larvae	1	4	—
Beetle, Coleoptera, terrestrial	1	—	—
water beetle—Dytiscidae	1	—	—
Bryozoa, statoblasts	—	3	—
Waterflea, <i>Daphnia</i> sp.	—	1	—
Amphipods, <i>Gammarus limnaeus</i>	—	3	—
Sow-bug, Isopoda	—	1	—
May-fly, Ephemeroptera, nymph	—	1	—
Damsel-fly, Odonata, nymph	—	1	—
Dragon-fly, Odonata, nymph	—	1	—
Insects, unidentified fragments	5	2	—
Gastropods, <i>Planorbis</i> sp.	8	2	—
<i>Physa gabbii</i>	1	—	—
<i>Physa</i> sp.	2	—	—
<i>Helisoma trivolvis</i>	2	1	—
Molluscs, unidentified fragments	10	1	—
Fish, carrion	1	—	—

sink in the muddy shallows where they are available for food in the following spring. In short, the bulrush is considered to be the plant of chief importance in the life of the lake. It is perhaps unnecessary to add that any loss of this growth through burning, which sometimes happens, completely disrupts the economy of the burned area for some years.

Cat-tail—*Typha latifolia*. The remarks in connection with the value of the bulrush as a cover plant and its use in nest building are applicable also to the cat-tail. The latter, however, does not produce a seed of value to waterfowl and for this reason is of less importance.

Sedges—*Carex* sp. Various species of sedge occupy the outer edge of the marsh areas and surrounding wet lands beyond the cat-tail and bulrush zone. These produce seeds of value and provide cover for nesting pond ducks.

Pondweeds; other submerged plants. The sago pondweed, *Potamogeton pectinatus*, is the commonest of the pondweeds; next in importance is *P. heterophyllus*, and third is *P. pusillus*. Their distribution, and also that of Water milfoil, *Myriophyllum spicatum*, Bladderwort, *Utricularia* sp., and Hornwort, *Ceratophyllum demersum*, is limited and becomes dominant only in the shallows at the north and south ends of the lake, where sand is an important constituent of the lake bottom. In these places there is usually an association of several of the species mentioned, forming a thick mass of growth. Duckweeds, *Lemna minor*, *Lemna trisulca*, and *Spirodella polyrhiza*, occur in sheltered areas within the bulrush and cat-tail marshes. All these plants form part of the diet of ducks.

Musk-grass—*Chara* sp. This alga is dominant over most of the lake bottom. In summer, as the water level lowers, changes in the appearance of the visible growth in the shallow areas rapidly take place. What formerly were *Chara* meadows become sodden masses as the tall growth collapses with the falling water. As the lake continues to fall and the *Chara* in the shallows becomes exposed to the sun, the upper surface of the mass dries and crumbles. Meanwhile the growth in deeper portions, always brittle and deciduous, contributes its surplus to the accumulation, so that eventually long islands of the material are strung along the shore outside the line of rushes. These are used as resting places by pond ducks.

*Chara* forms the chief item in the food of coots, and is important in the diet of some diving ducks. It provides also suitable habitat for the propagation of insects, crustaceans, and other small animals which are eaten by waterfowl. *Chara* oospores, which accumulate about the beds in the shallows, are eaten by pond ducks and to a lesser extent by diving ducks.

Filamentous algae—*Spirogyra*, *Zygnema*. In early summer these hair-like algae accumulate in thick mats on the surface of the water and later are deposited in windrows along open spaces on the shore, or become attached to the marsh growth as the waters recede. This growth is important in the ecology of the lake; while in the water it provides cover for many kinds of small organisms, and when dry it is used as nesting material by grebe and other birds.

### Conclusions

Observation of waterfowl at Swan lake, British Columbia, during a four-year period, and study of the stomach contents of 136 specimens, indicate

that competition for food between ducks and coots during the autumn months is negligible. The branches of *Chara* form the chief food of coots; the oospores of the alga and to a lesser extent the branches are eaten by some species of duck. As this is the most abundant growth in the lakes and is produced in unlimited quantities, there is sufficient for the requirements of a much greater coot and duck population than is ever likely to occur. It is probable that less than one per cent of the *Chara* crop is consumed by waterfowl. Seeds of *Scirpus* are most important in frequency of occurrence and percentage volume of duck food. Seeds of other aquatic plants and, to a lesser degree, molluscs and aquatic insects, also are eaten. These foods are inconspicuous items in the diet of coots.

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## STUDIES ON THE BIONOMICS AND CONTROL OF THE BURSATE NEMATODES OF HORSES AND SHEEP

### VII. THE EFFECT OF SOME SUBSTANCES, USED IN THE CONTROL OF FARM AND HOUSEHOLD PESTS, ON THE FREE-LIVING STAGES OF SCLEROSTOMES<sup>1</sup>

By I. W. PARNELL<sup>2</sup>

#### Abstract

The effect on the free-living stages of Sclerostomes caused by the addition to fresh horse faeces of some substances used in the control of household and farm pests, is discussed. Under the conditions of these experiments para- and orthodichlorobenzene will sterilize about 400 times their weight of faeces. Sodium fluoride will sterilize, on an average, approximately 150 times its weight of faeces, but it is almost twice as effective if applied as a very weak solution. Sodium silicofluoride, which also is most effective as a very weak solution, probably has an approximately equal value. Naphthalene, when mixed in the faeces, will sterilize about 270 times its own weight. Dichloropentanes will sterilize about 185 times their weight of faeces. 40% nicotine sulphate will, on an average, sterilize approximately 14 times its weight of faeces, but as a weak solution may be five or six times as effective. Ethylenedichloride, chloroform and carbon tetrachloride will sterilize about 21, 18 and 12 times their weight of faeces respectively. Trisodium phosphate will sterilize only about eight times its weight of faeces. Tobacco dust will probably sterilize slightly over twice its weight of faeces, but pyrethrum powder, derris powder and white hellebore powder have no lethal value. Ferric oxide and carbon monoxide also are useless.

This paper discusses the lethal effect of: paradichlorobenzene, orthodichlorobenzene, sodium fluoride, sodium silicofluoride, naphthalene, dichloropentanes, 40% nicotine sulphate, ethylenedichloride, chloroform, carbon tetrachloride, trisodium phosphate, tobacco dust, pyrethrum powder, derris powder, white hellebore powder, ferric oxide, and carbon monoxide, against the free-living stages of Sclerostomes in faeces, when the faeces were treated before the eggs could develop, according to the technique described in previous papers (22, 23).

Only under exceptional conditions is it probable that many of these chemicals could be used to control the free-living stages of Sclerostomes or related worms, in practice. However, it is hoped that some of the results may be of value in indicating some of the types and states of chemicals, which are, or are not, lethal to the free-living stages of this group of worms.

Dichloropentanes are practically the only chemicals of the above list that have been recommended, even tentatively, for use in Sclerostome control (16).

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Ethylenedichloride and carbon tetrachloride, and a mixture of the two at the rate of 3 : 1 by volume, have also been tested in horse faeces (16). The addition of from 0.5 to 0.8 cc. of dichloropentanes per 100 gm. of faeces reduced the number of larvae in the cultures to 0.0006% of the number in the controls, or less. When 0.7 to 0.9 cc. of ethylenedichloride and of carbon tetrachloride were added to the same quantity of fresh faeces the numbers of larvae were also very considerably reduced. Alone, carbon tetrachloride at the rate of slightly under 1 cc. per 100 gm. was effective; ethylenedichloride was probably slightly less effective. These three chemicals were apparently more effective against the eggs and pre-infective larvae than against the infective larvae.

Some of the chemicals that are discussed in this paper have been tested against nematodes that parasitize plants; in a few cases, in spite of the difficulty of comparing the results, they are suggestive.

Paradichlorobenzene at the rate of  $2\frac{1}{2}$  oz. per sq. yd. has failed against *Heterodera marioni* of tomatoes (24). A similar result was obtained against *H. schachtii* of potatoes in pots (1); however, on a larger scale, at the rate of 616 lb. per acre, an increased yield of potatoes resulted, although the nematodes were little affected (7). It has also been tested against fly larvae in manure at the rate of  $\frac{1}{2}$  lb. and 1 lb. per 10 cu. ft. of manure, but was not very effective (4). Against wireworms in soil the results with this chemical are contradictory (9). Its effectiveness against clothes moths, etc., is well known, and it is occasionally used by gardeners in greenhouses against slugs and snails. It acts on the nervous system of insects (5). The nervous system of Sclerostome larvae is very rudimentary, although sufficient to make them react quite quickly to various stimuli, such as light, heat, and touch. Orthodichlorobenzene has been tested less frequently.

The usefulness of sodium fluoride and sodium silicofluoride in farm practice is limited by their poisonousness to mammals. Sodium fluoride in solution of 1 and 2 lb. per gal. has been tested against fly larvae in manure; at the rate of 1 gal. to 1 cu. ft. of manure it was fairly effective (2). However, a 2% solution of sodium fluoride and a saturated solution of sodium silicofluoride had no effect after two hours on *Tylenchus dipsaci* (19).

Naphthalene also has been tested fairly frequently against plant nematodes and again the results are contradictory. Some workers have obtained no effect (1, 6, 13, 24); others have obtained slightly better results (17, 18). Against wireworms the results also are contradictory (9), but it is well known for its action against clothes moths.

Nicotine sulphate has been shown to be effective against fly larvae in manure, even as a 1 : 500 solution (4). As a constituent of sprays for fruit trees, as a fumigant for poultry lice, and as an anthelmintic, it is well known. Against free-living nematodes it has seldom, if ever, been tested.

Ethylenedichloride is more effective than carbon tetrachloride against *Tylenchus dipsaci*. If one part of the latter is mixed with three parts of the former, the fire hazard is reduced. Ethylenedichloride rapidly produces

anaesthesia, but a longer exposure (2 hr.) is necessary to cause the death of the nematodes (20). However, against nematodes in bulbs, ethylenedichloride was not really effective as a controlling agent (21).

Pyrethrum, derris, and hellebore powders are well known for their lethal action on certain insects. Against the leaf eelworm of chrysanthemums, pyrethrum and nicotine emulsions were more or less unsuccessful (15). Against fly larvae, hellebore is more effective powdered than ground (4), and as an infusion than as a powder (3).

The results obtained when ferric oxide has been applied to potatoes and oats have been very variable; under certain conditions the results have appeared to be beneficial (8, 12), and under others it has not been effective or has only temporarily stimulated the plants (7, 10, 11, 14).

### Results

Table I shows the values of the "controls" which are identified in Figs. 1 to 11 by Roman numerals.

TABLE I  
CONTROLS FOR CULTURES TABULATED IN FIGS. 1 TO 11

Series No.	Date cultures made	Days kept in C.T. room	Average number of larvae isolated	Series No.	Date cultures made	Days kept in C.T. room	Average number of larvae isolated
	1935				1937— <i>Conc.</i>		
XIII	7 May	32	11,000	CCLXIII	15 July	71	33,000
XXIX	12 July	20	19,500	CCLXV	19 July	67	18,000
XXXVII	6 August	35	38,000	CCLXVI	19 July	77	41,000
LIV	10 December	17	6,200	CCLXX	22 July	74	34,000
	1936			CCLXXVI	1 October	24	66,000
LXXIV	24 January	24	11,500	CCLXXVII	6 October	29	26,500
LXXXIII	3 March	13	25,000	CCLXXXIII	19 October	27	34,000
CXLI	9 July	24	10,000	CCLXXXIV	19 October	27	65,000
CLVII	28 October	37	43,000	CCXC	28 October	28	46,000
CLX	2 November	42	15,500	CCXCI	29 October	38	67,000
CLXVI	16 November	45	23,500	CCIC	16 November	42	53,000
CLXXII	24 November	51	35,000	CCCII	23 November	35	24,500
CLXXIII	26 November	60	37,000	CCCIII	24 November	44	32,000
CLXXXIV	17 December	56	52,000	CCCVI	2 December	36	63,000
CLXXXVI	28 December	49	42,000	CCCVII	3 December	35	58,000
	1937			CCCVIII	3 December	45	48,000
CCII	29 January	48	19,000	CCCIX	8 December	40	39,000
CCIII	2 February	52	27,000	CCCX	9 December	39	28,500
CCX	23 February	44	35,000	CCCXIII	20 December	38	34,000
CCXI	24 February	50	32,000		1938		
CCXIV	3 March	43	41,000	CCCXVIII	3 January	35	30,000
CCXV	3 March	47	45,000	CCCXXVII	1 February	37	35,000
CCXVIII	11 March	60	39,000	CCCXXVIII	2 February	36	29,000
CCXLI	18 May	27	49,000	CCCXXIX	3 February	35	40,000
CCXLII	18 May	27	35,000	CCCXXX	25 February	87	12,500
CCXLIII	18 May	27	91,000	CCCXXXI	7 March	77	19,500
CCVLI	1 June	23	66,000	CCCXXXVII	28 March	70	19,000
CCVLIII	1 June	23	85,000	CCCXXXVIII	28 March	84	14,500
CCLVIII	12 July	64	43,000	CCCLVI	6 July	51	38,000
				CCCLVII	7 July	50	32,000

*Paradichlorobenzene*

Paradichlorobenzene was tested both in the faeces and suspended in cheese cloth bags above them; Fig. 1 illustrates the results. Quantities of 0.01 to 8.0 gm. were applied. The cultures with the paradichlorobenzene above them showed that only about 1.7 gm. would evaporate in the containers, which have a capacity of nearly 550 cc. The results, like those obtained with some other chemicals that give off gases, were very irregular.

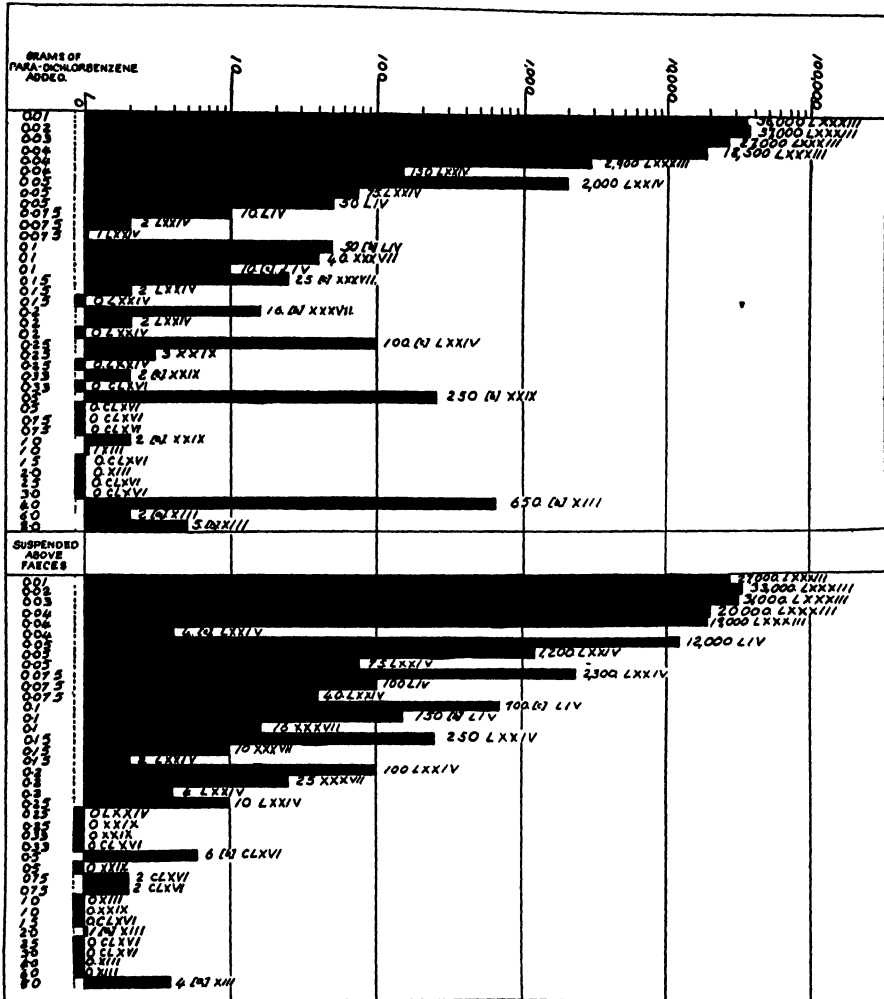


FIG. 1. Results of tests with paradichlorobenzene, mixed in and suspended above the 40-gm. cultures of fresh horse faeces. Roman numerals refer to the controls shown in Table I. In this, and in the subsequent figures, the letters have the following significance. a, all or practically all these *Sclerostome* larvae were dead; b, a considerable proportion of larvae were dead; c, a few larvae were dead; d, the culture included some *Sclerostome* larval sheaths or other debris, which was not counted; e, in the culture there were some live larvae, not counted, some or all of which were probably exsheathed *Sclerostomes* and which were sufficiently numerous to have put the culture in a significantly more numerous class, if they had been counted.

The number of larvae in one culture was considerably reduced by 0.04 gm. and in two cultures by 0.05 gm. mixed in the faeces. When 0.075 gm. was used, only 1, 2, and 10 larvae were recovered. With the addition of 0.1 gm. the larvae were slightly more numerous, but some were dead.

When suspended above the faeces, 0.04 and 0.05 gm. considerably reduced the number of larvae in one culture each, and 0.075 gm. reduced them in two cultures. From the cultures treated with 0.1 gm., 700, 150, and 15 larvae were isolated, but some were dead; 0.15 gm. reduced the larvae to 250, 10, and 2, while 0.2 gm. reduced them to 100, 25, and 4.

An average of these results suggests that under these conditions about 0.1 gm. or slightly less will sterilize the 40-gm. cultures of fresh horse faeces against *Sclerostomes*; this is equivalent to 0.25% of the weight of faeces.

### *Orthodichlorobenzene*

Orthodichlorobenzene, which has a specific gravity of 1.325, was tested in quantities of from 0.01 to 25.0 cc. Fig. 2 shows its sterilizing value. Again the results are very irregular. The number of larvae was considerably reduced by 0.033 cc. and the cultures were almost sterilized by 0.05 and 0.075 cc.; only four, three, and no larvae were recovered from the cultures treated with 0.1 cc. All the cultures treated with 0.15 and 0.2 cc. were free of larvae.

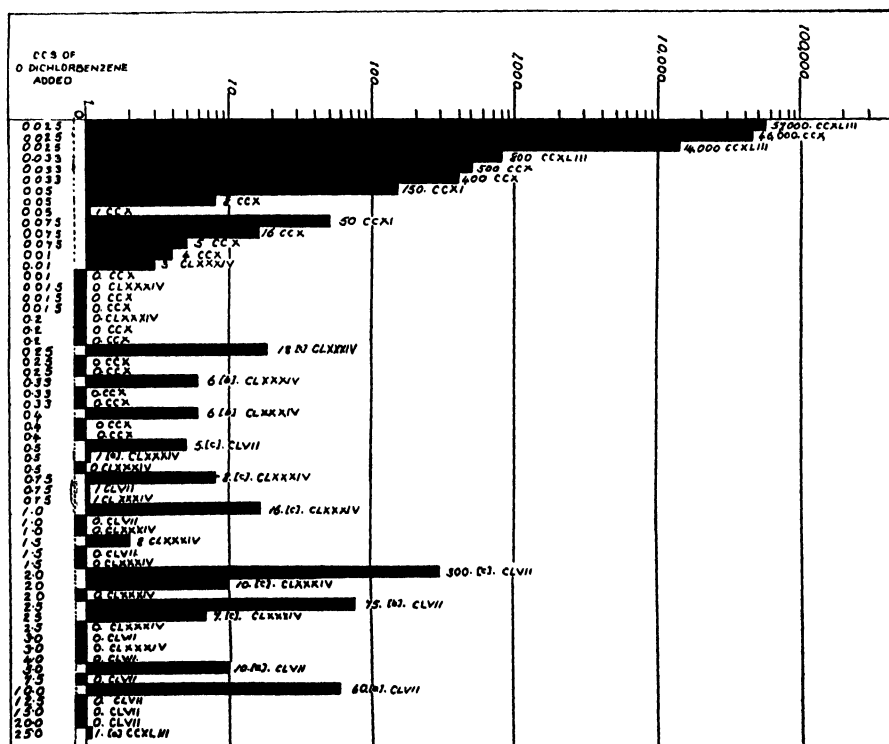


FIG. 2. Results of tests with orthodichlorobenzene.

Among the few larvae that were recovered from the cultures treated with larger quantities, the death rate was high. The amount of orthodichlorbenzene equivalent to about 0.25% of the weight of the treated faeces is 0.075 cc.

### Sodium Fluoride

Fig. 3 illustrates the results obtained with sodium fluoride when applied dry or in solution.

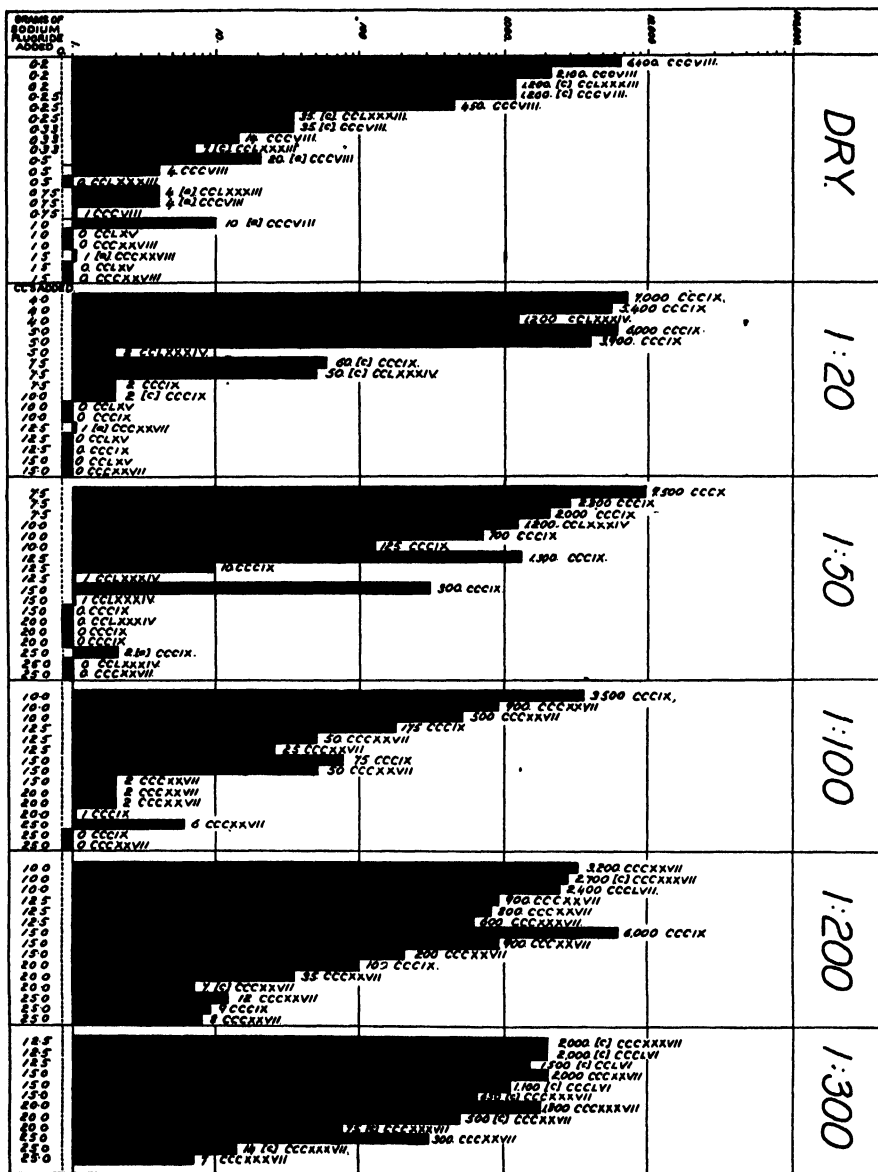


FIG. 3. - Results of tests with sodium fluoride, dry and in solution.

It was applied dry in quantities of 0.05 to 8.0 gm. When 0.2 gm. or less was applied, thousands of larvae survived. The numbers were considerably reduced by 0.25 gm.; 0.33 gm., or 0.82% of the weight of faeces, was practically effective as a sterilizing agent. No larvae were found in any of the cultures treated with 2.0 gm. or over.

As a 1 : 20 solution, quantities of 2.0 cc. and over were tested. With one exception, thousands of larvae were recovered from the cultures treated with 5.0 cc. or less; 7.5 cc. was practically and 10.0 cc. was completely effective. In 10.0 cc. of a 1 : 20 solution there is almost 0.5 gm. of sodium fluoride, equivalent to 1.25% of the weight of faeces.

As a 1 : 50 solution, which was tested in quantities of 4.0 cc. and over, 10.0 cc. made a very noticeable reduction in the number of larvae, while 12.5 cc. and 15.0 cc. both sterilized two out of three cultures; all the cultures were sterilized by 20.0 and by 25.0 cc. In 15.0 cc. of a 1 : 50 solution there is approximately 0.3 gm. of sodium fluoride, or 0.75% of the weight of faeces.

Applied as a 1 : 100 solution, 10.0 cc. again considerably reduced the number of larvae; 12.5 and 15.0 cc. reduced them further, and the latter quantity sterilized one culture. Sterilization was almost complete when 20.0 and 25.0 cc. were applied; the former quantity contains 0.2 gm., or 0.5% of the weight of faeces.

Sodium fluoride was also tested as a 1 : 200, 1 : 300, 1 : 400, 1 : 500, and 1 : 600 aqueous solution. Fig. 3 shows that the greater quantities of fluid of both the 1 : 200 and 1 : 300 solutions caused a marked reduction in the number of larvae; the other solutions, which are not illustrated, had similar, but less marked, effects on the numbers. The 1 : 300 and 1 : 400, like the 1 : 200, solutions, tended to cause the death of a few of the larvae after they had reached the third stage. In 25.0 cc. of a 1 : 200 solution there is about 0.125 gm. of sodium fluoride and only about 0.083 gm. in the same quantity of fluid of a 1 : 300 solution; these quantities are equivalent to 0.31% and 0.21% of the weight of faeces. If the different quantities of sodium fluoride that are required to cause sterilization, when applied dry and as solutions of various strengths, are averaged, a value of approximately two-thirds of one per cent by weight is obtained. However, sodium fluoride is more effective when applied as a very weak solution.

#### *Sodium Silicofluoride*

The effects of sodium silicofluoride on the number and condition of the third stage Sclerostome larvae recovered from faeces are illustrated in Fig. 4.

The action of sodium silicofluoride is similar to that of some of the sulphur and chlorine salts (which will be described in subsequent papers of this series), in that it does not kill many of the larvae until they reach the third stage.

Sodium silicofluoride was tested dry in quantities of 0.05 to 8.0 gm. In one of the cultures in which 0.1 gm. was mixed, a few larvae died after reaching the third stage; in all the cultures in which 0.15, 0.2 or 0.25 gm. was mixed,



from the cultures treated with 6.0 gm. or over. Since there seems to be no practical method of determining whether the larvae that reached the third stage and then rapidly died would ever have been infective to a suitable host, it is extremely difficult to be certain of the exact effective percentage of dry sodium silicofluoride.

Fig. 4 shows that while there is a distinct tendency for some of the larvae to reach the infective stage and then die when the faeces are treated with sodium fluoride in solution, the tendency is not so marked as when this chemical is applied dry.

Sodium silicofluoride was tested as a 1 : 100 aqueous solution, but at that dilution some sediment remains. The results were irregular; however, since one culture was sterilized by 15.0 cc. and two completely and one almost sterilized by 20.0 cc., it is probable that about 20.0 cc. is the amount required to cause sterilization. This contains 0.2 gm., equivalent to 0.5% of the weight of faeces.

When applied as a 1:200 or as a 1:300 aqueous solution, the exact interpretation of the results again is difficult. When a 1:200 solution was applied, one of three cultures was sterilized by 15.0 cc., and two each by 20.0 and 25.0 cc. When added as a 1 : 300 solution, there were numerous larvae in two cultures treated with 20.0 cc., but 25.0 cc. was effective. If the results are averaged it seems probable that slightly under 25.0 cc. of a 1 : 200 solution and 25.0 cc. of a 1 : 300 solution, or about 0.3% and 0.21% are effective.

Sodium silicofluoride was also tested in 1 : 400, 1 : 500, 1 : 600, and 1 : 800 aqueous solutions. The larger quantities of fluids of all the solutions caused a marked reduction in the number of larvae that reached the third stage; in the stronger of these solutions there was also a tendency for these larvae to die.

If it is considered that 0.33 gm. of dry sodium silicofluoride is effective in sterilizing the 40-gm. cultures, the average sterilizing value of this chemical is slightly under half of one per cent. However, this value may perhaps be too high. This chemical also is most effective when applied as a very weak solution.

### *Naphthalene*

Naphthalene was tested in the faeces and suspended above them. The results obtained are shown in Fig. 5. Quantities of 0.01 to 8.0 gm. were applied.

When the naphthalene was mixed in the faeces the results were more regular. When 0.05 gm. or less was added thousands of larvae survived; in two of the three cultures treated with 0.075 and with 0.1 gm., the number of larvae was considerably reduced. When 0.15 gm. (equal to 0.37%) or more was mixed in the faeces, the cultures were effectively sterilized.

When the naphthalene was suspended above the cultures, it was found that from 0.3 to 1.3 gm. evaporated. One of three cultures was sterilized by 0.1, 0.15, 0.2, and 0.25 gm.; two cultures were sterilized by 0.33 gm., but

thousands of larvae were recovered from other cultures treated with these or with smaller quantities. When 0.5 gm., equivalent to 1.25%, or more was suspended above the faeces they were sterilized against Sclerostomes.

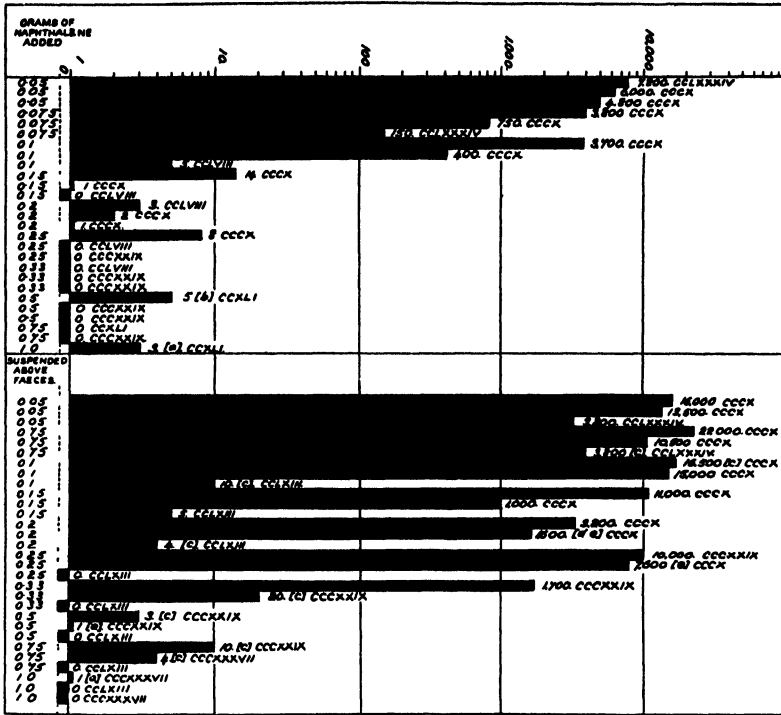


FIG. 5. Results of tests with naphthalene, mixed in and suspended above the cultures.

### Dichloropentanes

Dichloropentanes, which have a specific gravity of 1.07, were tested in quantities of 0.02 to 25.0 cc. In Fig. 6 are shown the numbers and condition of the Sclerostome larvae which were recovered from the cultures treated with it.

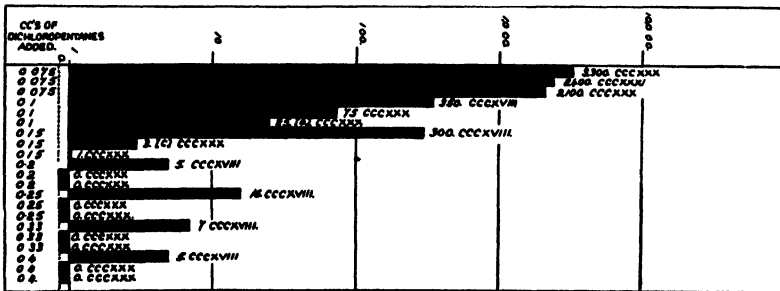


FIG. 6. Results of tests with dichloropentanes.

Thousands of larvae were recovered when 0.075 cc. or less was mixed in the faeces. When 0.1 cc. was added, the number of larvae was very considerably reduced and 0.15 cc. sterilized two out of three cultures. The addition of 0.2 cc. (equal to 0.54% of the weight of faeces) or larger quantities, sterilized them against *Sclerostomes*.

#### *40% Nicotine Sulphate*

Fig. 7 illustrates the effect on the *Sclerostome* larvae of mixing 40% nicotine sulphate, both undiluted and in solution, in faeces. It has a specific gravity of 1.19.

This substance was applied undiluted in quantities of 0.2 to 15.0 cc.; in all cultures treated with 1.5 cc. or less, thousands of larvae reached the third stage; however, in a few cultures some of the larvae subsequently died. When 2.0 cc. was added, 900, 700, and 125 larvae were recovered; 2.5 cc. further reduced the numbers to 500, 300, and 60. From one culture treated with 3.0 cc., 1,000 larvae were isolated, but from the other two cultures only 35 and 30 larvae were obtained; most of the former and all of the latter were dead. The addition of 4.0 cc. practically sterilized the cultures and from the three cultures treated with 5.0 cc. only one larva was obtained. However, from two of the three cultures treated with 7.5 cc., 300 and 50 larvae were obtained, most of the larvae from the former and all from the latter culture being dead; the third culture was free of larvae, as were the cultures treated with larger amounts. If the results obtained are averaged it seems probable that approximately 4.0 cc., or 11.9% by weight of undiluted 40% nicotine sulphate will cause sterilization.

Diluted with twice its volume of water, this substance was tested in quantities of 0.4 to 20.0 cc. When 2.0 cc. or less was applied, thousands of larvae were recovered, including a few that were dead. The results with quantities of 2.5 to 7.5 cc. were irregular. One culture was sterilized by 2.5, 3.0, and 4.0 cc. and in the other culture treated with these quantities there were decreasing numbers of larvae; however, there was a slight increase in numbers in the cultures to which 5.0 cc. was added. The addition of 7.5 cc. was almost effective; greater quantities were uniformly effective. At a dilution of 1 : 2, 7.5 cc. is equivalent to 7.4% by weight.

When diluted with four times its volume of water, the larvae were numerous after treatment with 3.0 cc. or less. When 4.0 cc. or more was added, the number of larvae was considerably reduced, but 15.0 cc., or 8.9%, was necessary to effect complete sterilization. When diluted with eight times its volume of water it was more effective, and 10.0 cc. and over caused sterilization, except in one culture treated with 15.0 cc. Moreover, in the cultures treated with 4.0, 5.0 and 7.5 cc., there was a very marked reduction in the numbers of larvae recovered, and a few cultures were almost sterilized. When diluted at the rate of 1 : 8, 10.0 cc. is equivalent to 3.3% by weight.

Applied in a dilution of 1 : 20 the results again were most irregular; quantities of 7.5 cc. and over caused a considerable decrease in the numbers of larvae, including the sterilizing of one culture by 10.0 cc. and two out of three

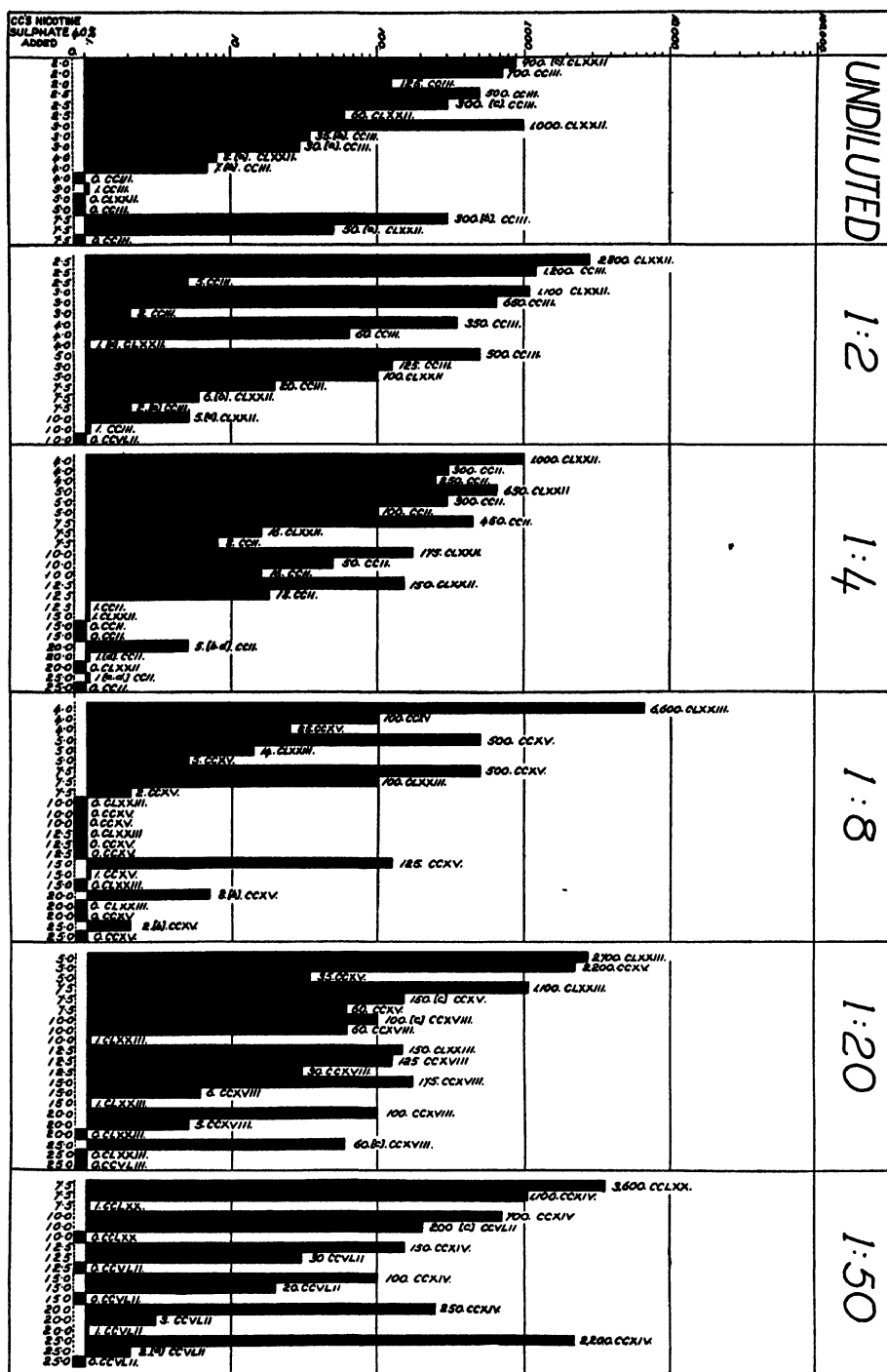


FIG. 7. Results of tests with 40% nicotine sulphate, undiluted and in solution.

cultures were sterilized by 15.0, 20.0 and 25.0 cc.; the latter quantity contains the equivalent of 3.5% by weight.

Diluted with 50 times its volume of water the results were equally irregular. One culture was sterilized by 7.5, 10.0, 12.5, and 15.0 cc., and two each by 20.0 and 25.0 cc.; in the other cultures treated by 10.0 cc. and over, the number of larvae was considerably reduced, although from the other cultures treated with 20.0 and 25.0 cc., 250 and 2,200 larvae were recovered. In 25.0 cc. there is the equivalent of 1.46% of 40% nicotine sulphate.

Nicotine sulphate was also tested diluted with 100 and 200 times its volume of water. One culture was sterilized by 15.0, 20.0, and 25.0 cc. of the stronger solution, but in the other cultures there were thousands of larvae.

The results described above suggest that nicotine sulphate is most effective as a medium strength solution, and that the average amount required (of the 40% grade) is about 7% of the weight of faeces.

### Ethylenedichloride

Ethylenedichloride, the results with which are illustrated in Fig. 8, has a specific gravity of 1.25.

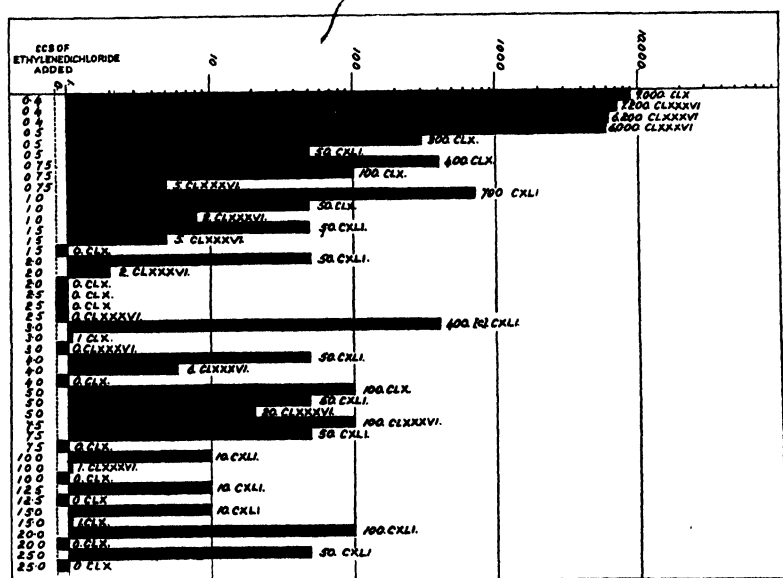


FIG. 8. Results of tests with ethylenedichloride.

It was tested undiluted in quantities of 0.25 to 25.0 cc. The results obtained were very irregular. In two of the cultures treated with 0.5 cc. and in all the cultures treated with larger quantities, the number of larvae was considerably reduced, but from many of the cultures treated with up to 25.0 cc. some active larvae were recovered. On an average, the figures suggest that about 1.5 cc. of ethylenedichloride, equivalent to 4.7% of the weight of treated faeces, will sterilize them.

This chemical was also tested as a 1 : 100 aqueous "solution" and as a 1 : 200 aqueous solution, but even when 25.0 cc. was applied several thousand larvae survived.

### Chloroform

Fig. 9 shows the results obtained with chloroform. It has a specific gravity of almost 1.5.

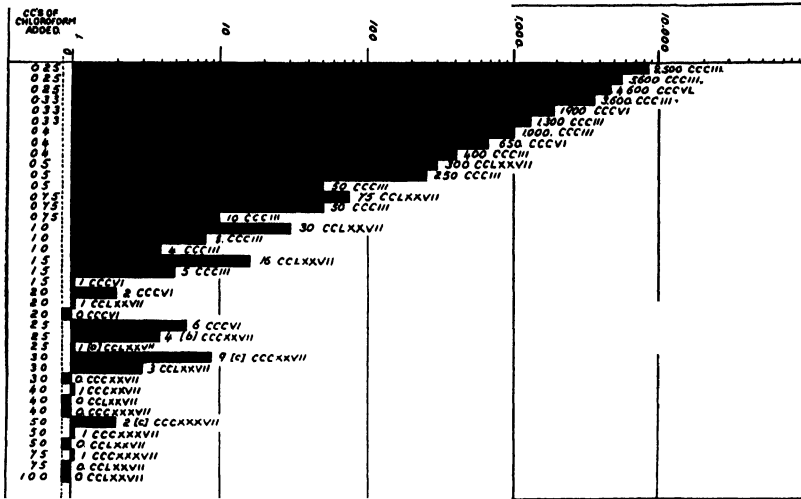


FIG. 9. Results of tests with chloroform.

Chloroform was mixed in the faeces in quantities of from 0.1 to 25.0 cc. When 0.33 cc. or less was added, thousands of larvae were recovered from the cultures. With the addition of 0.4 cc. or more, the number of larvae was considerably reduced and became progressively less; however, three and five larvae were recovered from the cultures treated with 15.0 and 20.0 cc. The results indicate that about 1.5 cc., or by weight 5.6%, will sterilize the cultures against *Sclerostomes*. Chloroform was also tested as a 1:200 aqueous "solution" but was quite ineffective.

### Carbon Tetrachloride

The effect of adding carbon tetrachloride to faeces is illustrated in Fig. 10. This chemical has a specific gravity of 1.58. It was added to the cultures in quantities of 0.1 to 25.0 cc. From one culture treated with 0.5 cc., from two with 0.4 and 0.25 cc., and from all cultures treated with 0.33 and 0.2 cc. or less, thousands of active third stage larvae were recovered; from the other cultures the number of larvae was smaller. The addition of 0.75 cc. and over considerably reduced the numbers of larvae, but the results suggest that about 2.0 cc. is necessary to effect sterilization. This quantity is equivalent to just under 8% of the weight of treated faeces.

### Trisodium Phosphate

Fig. 11 illustrates the results obtained with trisodium phosphate, which was tested both dry and in solution.

Dry, it was tested in quantities of 1.0 to 10.0 gm. When 3.0 gm. or less was added, thousands of larvae survived; the addition of 4.0 gm. not only considerably reduced the number of larvae that was recovered, but caused a heavy death rate among those which were collected. No larvae were recovered from any of the cultures to which 5.0 gm., or 12.5%, was added, but 175, 75, and 2 larvae, all of which were dead, were recovered from one of each of the cultures treated with 6.0, 7.0, and 8.0 gm.

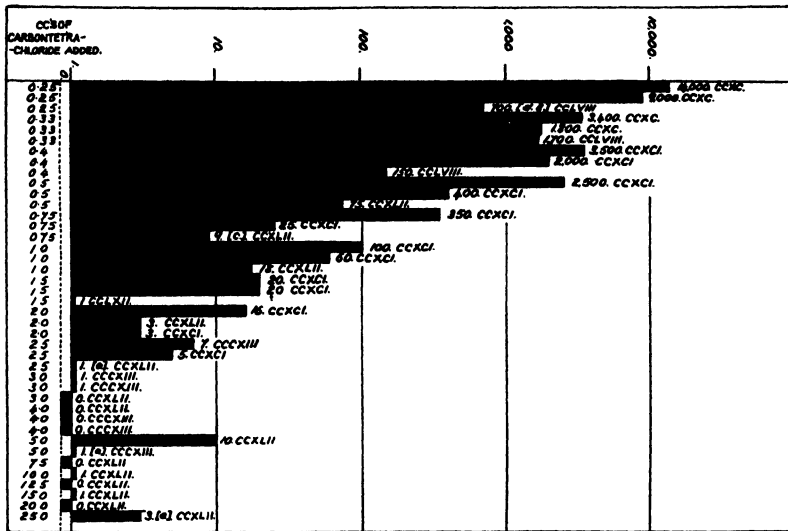


FIG. 10. Results of tests with carbon tetrachloride.

Trisodium phosphate was applied as a 1 : 2 aqueous solution in quantities of between 2.0 and 25.0 cc. One culture was sterilized by 10.0 cc., but from the other two treated with this amount or less, thousands of larvae were recovered. The addition of 12.5 cc. greatly reduced the number of larvae and killed the great majority of those recovered. The addition of 15.0 cc. and over sterilized the cultures; in this quantity of fluid there is almost 6.0 gm. of trisodium phosphate, or 15% of the weight of the treated faeces.

Applied as a 1 : 4 aqueous solution, the addition of 12.5 cc. reduced the numbers of larvae in two cultures to 400 and 1,200, and slightly lowered the viability of the larvae in all cultures. Two out of three cultures were practically sterilized by 15.0 and by 20.0 cc.; all were sterilized by 25.0 cc. In the latter quantity there is 6.0 gm., while in 20.0 cc. there is 4.8 gm. or 12% of the weight of treated faeces.

This chemical was added to the cultures as a 1 : 8 aqueous solution in quantities of 5.0 to 25.0 cc. When 15.0 cc. or less was added, thousands of larvae were found. The number of larvae was very considerably reduced in two out of three cultures by 20 cc. The addition of 25.0 cc., containing about 3.0 gm., sterilized one culture, from another 250 larvae were recovered, and from the third 1,500 larvae, but a considerable proportion of these were dead.

A 1 : 20 aqueous solution was also tested, but even 25.0 cc. only reduced the number of larvae to 9,000 and 8,500.

These results suggest that this chemical would have to be added at the rate of about 12.5% by weight to sterilize the faeces against *Sclerostomes*.

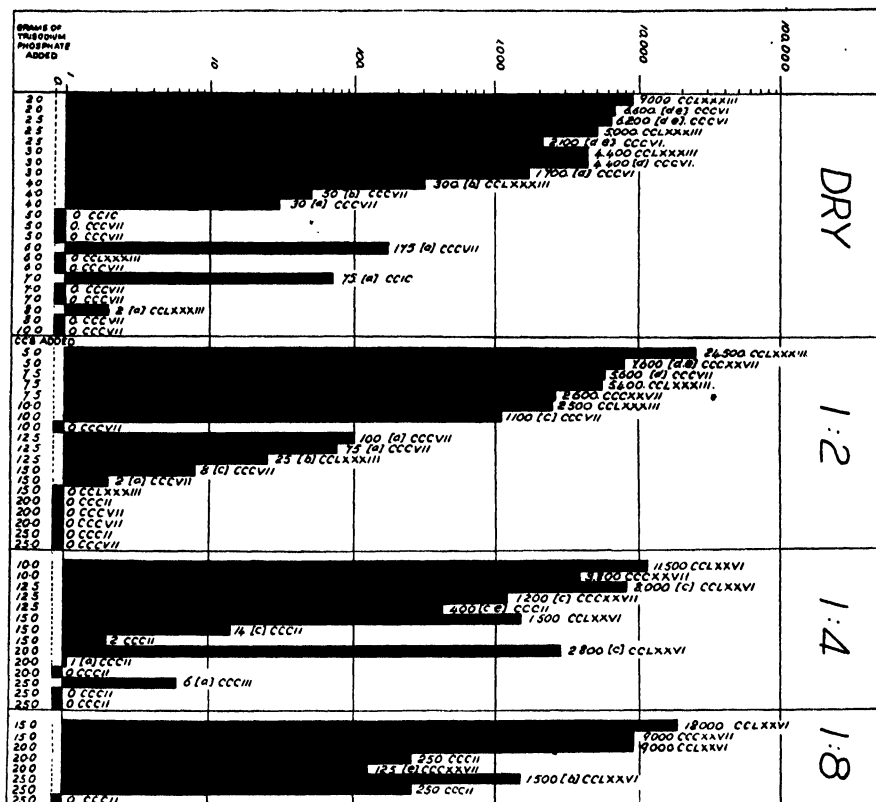


FIG. 11. Results of tests with trisodium phosphate, dry and in solution.

### Tobacco Dust

Tobacco dust was added dry in quantities of 1.0 to 20.0 gm. The results were irregular and are not illustrated. The number of larvae in one of three cultures was considerably reduced by 4.0, 5.0, and 7.0 gm.; one culture was sterilized by 6.0 gm. The larvae in two out of three cultures were much reduced in numbers by 8.0 and 10.0 gm. The addition of 12.0 gm. sterilized two cultures, and from the third only 150 larvae, including a few dead, were recovered. However, from the cultures treated with 14.0 gm., 5, 200, 250 including many dead, and 8 larvae were isolated. Both 16.0 and 20.0 gm. had the effect of reducing considerably the number of larvae in one culture and sterilizing the other two. These results suggest that the addition of 50% or slightly less of tobacco dust to faeces sterilizes them against *Sclerostomes*.

Tobacco dust was also tested as an infusion with four times its weight of water; it showed no indication of being effective.

#### *Pyrethrum, Derris, and Hellebore Powders*

Pyrethrum powder, derris powder, and white hellebore powder were tested dry, in quantities of 1.0 to 20.0 gm. and as infusions with four times their weight of water in quantities of 2.0, 5.0, 10.0, 15.0, and 20.0 cc. In no case did they have any lethal value.

#### *Ferric Oxide*

Brown ferric oxide was applied dry in quantities of 1.0 to 20.0 gm. In the first series of cultures, the largest quantities of ferric oxide appeared to reduce the numbers of larvae, but the confirmatory series showed that this chemical has no lethal value.

#### *Carbon Monoxide*

Carbon monoxide was tested by displacing the air in the containers with as much as 500 cc. of gas. The gas was made by dripping formic acid on to warm sulphuric acid, and was collected in a large container, from which it was slowly displaced into the bottom of the vessels containing the cultures. On some of the containers rubber rings were left to retain the gas for 48 hr., 7 days, 14 days, and until the cultures were rebagged a few days before the extraction of the larvae.

Carbon monoxide did not sterilize any cultures with no rubber ring on the jars or on which the ring was left for only 48 hr. Six out of eight cultures were almost or completely sterilized when the rings were left on for 7 or 14 days. But two controls on which the rings were left for 7 and 14 days respectively yielded only 31 and 5 larvae. Furthermore, 12 control cultures were left with the rings on until a few days before the extraction of the larvae; nine yielded no larvae, and from the other three, 25 dead, 40, and 27,000 larvae were obtained. Eleven cultures, treated with 25 to 500 cc. of carbon monoxide, with the rings left on until a few days before the extraction of the larvae, were also free or almost free of larvae. Some of these cultures were kept in the light, others in the dark; no difference was noted.

As 953 control cultures have been made in jars without rubber rings, of which only one has been free of larvae and only three contained under a hundred larvae, it seems evident that it was not the carbon monoxide which sterilized the faeces in these experiments. Probably the cause was the gases generated by the faeces, perhaps associated with lack of oxygen. It seems possible that further work, and tests that are being made in large wooden containers holding nearly a cubic yard of manure, may show that this phenomenon could be used in practice.

### **Conclusions**

The results discussed in this paper show that some chemicals that give off gases are extremely lethal to Sclerostomes, but that others, known to be lethal to other forms of animal life, have a comparatively low lethal value or are useless against Sclerostomes; that some substances, known to be lethal

to some forms of animal life, are useless against Sclerostomes, and that some chemicals, although extremely lethal to Sclerostomes, when added to fresh faeces, do not kill many of the larvae until they reach the third stage.

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## OBSERVATIONS ON THE BIONOMICS OF OVA AND MIRACIDIA OF *FASCIOLA HEPATICA* LINN., IN EASTERN CANADA<sup>1</sup>

BY HENRY J. GRIFFITHS<sup>2</sup>

### Abstract

The effect of temperature, light, darkness, and chemicals on the hatching of the eggs of *Fasciola hepatica* is discussed. Some malformations of the eggs of this parasite together with observations on the miracidia are recorded. A description of methods employed for attempted infestation of suspected snails is given. In no instance, however, were any specimens of the 11 species of snails exposed, proven to act as intermediate host for *F. hepatica*.

### Introduction

During an investigation of the incidence of *Fasciola hepatica* Linn. in the lower St. Lawrence valley, a number of observations was made on the bionomics of the miracidia and ova of this parasite. The results, together with the findings of other investigators, are presented in the following paper.

In order to carry on experimental tests on the ova of *F. hepatica*, it is highly desirable that a readily accessible supply of material be available. It is preferable to have pure cultures of eggs direct from the gall bladder of the infected host. The contents of the gall bladder should be emptied into a glass container and the eggs allowed to sediment in water. After 15 min. the supernatant fluid may be poured off and the jar refilled with water. This procedure should be carried on until all traces of bile are removed.

The eggs may then be left for further development or held in a refrigerator at 2 to 4° C. until development is required. Ova may be obtained directly from the faeces of a heavily infected individual by screening, sedimenting and washing to remove all soluble matter; this method, however, is very tedious unless the host animal is very heavily infected.

### Effects of Temperature, Light, Darkness, and Chemicals on the Hatching of Ova

In the course of his classical research on the life cycle of *F. hepatica*, Thomas (9) found that temperature played an important role in controlling the rate of development of the eggs. The optimum temperature was about

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22 to 26° C., at which development took about 14 days; at 16 to 18° C., development took two or three months, while none took place at less than 10° C. The influence of temperature on the rate of development of fluke eggs is of practical importance, since it probably may be considered as a principal factor in determining when seasonal infestation of molluscs occurs, and the probable period of infection of the definitive host as a result of escaping cercariae.

Ross and McKay (6) recorded their first hatch after eight days at 26 to 27.5° C.; at 25° C. the mass hatch took place on the tenth day, and no development took place below 10° C. Mattes (4) stated that eggs develop only on addition of water and above 9° C., development being slow between 9 to 15° C. Development to miracidia, he noted, required two weeks at 20 to 30° C. Shaw and Simms (7) found that ova hatched at varying periods under room conditions, some in as short a time as 14 days; however, some cultures continued to hatch at intervals up to 13 months and 20 days after collection.

From a comparison of results obtained on hatching with those of other investigators, it is apparent that the times of hatching with relation to temperature may show wide variations. At uniformly high temperatures, Ross and McKay (6) obtained a hatch in eight days, with a temperature range of 20 to 30° C.; Mattes (4) found that development to miracidia requires two weeks at 20 to 30° C., 20 to 40 days at 17 to 18° C., the optimum being 20 to 25° C. In the author's observations, ova were seen to hatch in 13 to 15 days, at a temperature of 20° C., the mass hatch usually occurring at about three weeks.

A factor of major importance and interest to be considered, with the severe winter temperatures experienced in Canada, is the effect of low temperatures on ova. Shaw and Simms (7) found that if newly collected ova and well embryonated ova were exposed to a temperature of -12° C. for 24 hr., newly collected ova hatched after thawing, but those containing live embryos at the time of exposure were destroyed. Because of the author's limited supply of ova, it was necessary to keep them in the refrigerator at 2 to 4° C. The ova were placed in the refrigerator in November 1933, and until March 1935 these gave a supply of miracidia when required, hatching usually within three or four weeks after removal to room temperatures or 20° C. After a period of five months, on removal from cold storage to a temperature of 20° C., ova were found to hatch in 16 days. After a period of 11 months, hatching results were still satisfactory, though the percentage hatch appeared to be slightly decreasing. After 16 months, hatching results were still satisfactory, the ova hatching in from 24 to 30 days after removal from the refrigerator.

It does not appear that the cold storage of ova up to 16 months has any material effect on their ability to hatch. However, the vitality and penetration power of the miracidia might be questioned. This point could not be investigated owing to the fact that a proven intermediate host was not available for exposure to attack by these miracidia. Krull (2) found that eggs held

at approximately 2 to 10° C., for a period of two years, six months, and seven days, upon removal to room temperature, developed and hatched readily in 18 days. These miracidia were used for infestation of laboratory-raised *Pseudosuccinea columella*, which were infected en masse, with positive results.

Shaw and Simms (7) have shown that embryonated ova are destroyed on exposure to -12° C. for 24 hr. To observe the effect of cold on embryonated ova, a culture of ova, just commencing to hatch, was placed in the refrigerator at 2 to 4° C. Hatching was checked, and it was found that for some time following, ova could be removed from this to ordinary room temperature and would almost immediately commence to hatch; the miracidia upon hatching appeared quite normal.

After 10 days at 2 to 4° C., on removal to room temperature, some of the ova hatched within a very few minutes, the miracidia appearing normal. After 14, 37 and 168 days, a number of ova hatched immediately upon removal to room temperature, the embryos within the egg at the longest period having the appearance of being slightly shrunken, but still hatching normally. After a period of nine months, ova were removed to room temperature and a good hatch was obtained, but signs of lack of motility and activity of the miracidia were apparent. After 14 months storage, hatching was slowed considerably, the first ova hatching in 35 min. The mass hatch did not occur until one hour after removal from the refrigerator; only a comparatively small percentage hatched, the embryos appearing to have disintegrated to some extent. These miracidia showed considerable lack of motility, the cilia not functioning normally and the organism moving slowly in a rotary manner.

Darkness did not appear to influence the development of ova to any marked degree, though some delay in hatching was evident. It was noted that after three or four weeks, cultures kept in darkness would usually yield miracidia on exposure to light. The keeping of cultures in darkness was consequently a convenient means of obtaining miracidia when desired.

From the work of Ross and McKay (6) there is no indication that the application of copper sulphate in the field, for mollusc control, would in any way effect the hatchability of fluke ova. They exposed ova to such dilutions of copper sulphate in water as had been found lethal to intermediate hosts, viz., 1 : 1,000, 1 : 10,000, 1 : 100,000 and 1 : 1,000,000. The eggs were exposed to these concentrations for 24 hr., washed, incubated, and found to develop satisfactorily. Luhrs (3), however, claims that sea water has a marked lethal effect on ova, destroying them in 16 days, the miracidia being destroyed in 45 sec.

In order to observe the effect on fluke ova of a few chemicals that might be utilized advantageously on certain pastures, a number of cultures was made using solutions of different chemicals in water. These cultures were maintained at room temperature at about 20° C. Ova cultured in 1% and 5% copper sulphate respectively, showed no signs of development. In 5% potassium dichromate the ova collapsed rapidly, and in 1% sulphate of ammonia

development to the embryo stage was observed, a few ova hatching, but perishing on contact with the media. In 1% kainit, the ova developed to an advanced stage, but perished in the shell prior to hatching.

It seems apparent that in order to prevent ova development by the use of desirable chemicals, exposure to a strong solution for a considerable time would be necessary, a measure that would be impracticable in the field.

By frequently changing the water of cultures, the rapidity of development of the ova seems to increase to a certain degree, the influencing factor probably being the presence of available oxygen. Mattes (4) states that foul water inhibits the egg development and may even kill the ova.

On several occasions, stock storage jars of *F. hepatica* ova were found to be infected with a parasitic fungus. While it did not resemble *Catenaria anguillulae*, a Chytridiacean parasite of *F. hepatica* ova described by Butler and Buckley (1), identification was not possible because the fungus could not be induced to fruit. This parasite may possibly have been responsible for a considerable decline in the percentage hatch of ova kept at 2 to 4° C., though this would not seem to be an optimum temperature for its development.

#### SOME MALFORMATIONS OF *F. Hepatica* OVA

During the course of routine examination of *F. hepatica* ova the opportunity of observing malformed eggs was presented. The eggs in question were from a rabbit harbouring 13 adult flukes, the metacercariae of which had been obtained from snails shipped from Oregon.

Taylor (8) refers to the production of malformed eggs by *F. hepatica* and considers it to be a normal occurrence in the early stages of activity of the generative organs of the liver fluke. The ova examined by Taylor were collected from a sheep that had died from sub-acute fascioliasis, and the majority were observed to be smaller than usual, of a darker colour, and bearing a protuberance at one end. The malformed ova observed in this investigation were obtained from a rabbit infested some nine months previously. A considerable variation in egg size was found, tending to range below the usual sizes recorded. Egg shapes were very irregular and many ova were infertile.

The malformation of greatest interest was the appearance of many ova surrounded with what superficially resembled a uniform gelatinous coating, bearing, in a few cases, a distinct spine on one side of the ovum. The average length of the ovum plus the coating was 180 $\mu$ , the breadth averaging 121 $\mu$  as compared with the usual measurements of 130 to 145 $\mu$  long by 70 to 90 $\mu$  wide. The coating varied in thickness from 20 to 25 $\mu$ . Proof is lacking that the coating was gelatinous, but on addition of a little formalin, the ovum collapsed and the coating assumed an elastic condition and a coagulated opaque appearance, which might suggest that some substance of a gelatinous nature was present.

## The Miracidia

The period of free activity of the miracidia of *F. hepatica* is a comparatively short one, in many cases being only a few hours. The usual time of hatching of ova cultures was consistently observed to be in the late morning. This may possibly have been due to a temperature change or an increase in the intensity of light in the laboratory. Exposure of well-embryonated ova to bright artificial illumination did not influence the rate of hatching and could not be used as a means of inducing liberation of miracidia. Only on two occasions were miracidia observed to emerge backwards from ova at the time of hatching.

When the development of the miracidium is completed it does not necessarily hatch, but may remain for some time within the egg shell. Addition of cold water has been known to cause hatching, but it is not known whether the temperature change or some more complex factor is responsible. Mattes (4) claims that pH is a very important factor in the hatching of ova. He states that increase of acidity from pH 8.0–8.5 to 5.5–6.0, either by use of naturally acid water or by acidifying the culture water with acids, causes hatching in from 10 to 15 min. Greater acidities (pH 3.0 or lower) are ineffective, while in alkaline water (pH 7.5 or higher) the miracidia fail to hatch and eventually die within the shells. Mattes further states that lowering of temperature without increased acidity is ineffective, and that in nature rain is probably the factor that raises the acidity. This causes hatching, owing to the fact that a favourable acidity increases activity of the flame cells and the general activity of the miracidia inside the shell. The actual opening of the operculum is caused by intake of water into the fluid vacuole.

Shaw and Simms (7) found that miracidia would live for 24 hr. after hatching, but the majority died after 8 hr. Ross and McKay (6), having observed that eggs hatching in the spring yielded miracidia that lived longer than those hatching in the summer, carried out experiments to ascertain whether temperature influenced longevity. Miracidia, after hatching, were kept at temperatures varying from 8 to 26° C. Those kept at low temperatures were active for 48 hr., and some individuals remained active for 72 hr. Those at the higher temperatures however, were all dead within 8 hr., temperatures between these extremes providing corresponding periods of longevity.

From the foregoing data, it seems that the probable periods of maximum infection of the molluscan hosts in Canada would be in the spring or fall. Lower water temperatures and resultant increased longevity of miracidia would permit greater possibility of locating the required intermediate host.

During the early part of this investigation, observations were made of the reactions of the miracidia to the molluscs collected from the field. In order to permit detailed observations the larger species were exposed to the miracidia in a Syracuse watch glass, but the smaller snails could be observed conveniently under the binocular microscope in the concavity of a hanging-drop slide. The

desired number of miracidia was admitted by means of a glass pipette, having previously been taken from a culture at the peak of its hatching period.

A further method of exposure was to place a known number of embryonated ova in a glass vial, suspend this vial below the water surface in the aquarium tank by attachment to a cork, and allow the miracidia to hatch and be liberated as they would under natural conditions. This method was found very suitable because the desired number of ova could be used according to the number of snails in the tank, and over-infestation was unlikely to occur. Furthermore, the glass vial could be removed and examined to ascertain how many of the ova had hatched.

In large aquarium tanks when no individual observations were made, miracidia or embryonated ova were admitted in such numbers as would be unlikely to occur under field conditions. These mass infestations were repeated at intervals to permit every opportunity of infection of the molluscs. A further means of exposure employed was to add infected faeces direct to the tank and thus ensure a hatch under as near field conditions as possible.

#### *Behaviour of Miracidia in Presence of Molluscs*

During the course of this investigation, 11 species of molluscs from the lower St. Lawrence valley were exposed frequently to miracidia, but none of these was implicated as the intermediate host of *F. hepatica*. Several species did not possess close relationship to the Lymneae, to which the suspicion of vector of this fluke is usually attributed.

To Mr. A. La Rocque of the National Museum of Canada, and Dr. F. C. Baker of Illinois, the author is greatly indebted for the identification of the following species of snails:—

*Helisoma infracarinatum* (Baker)

*H. antrosom* (Say) var. *unicarinatum*

*Stagnicola palustris elodes* (Say)

*S. palustris* var.

*Physa gyrina* (Say)

*Amnicola* sp.

*Fossaria obrussa* (Say)

*F. obrussa exigua*

*F. umbilicata*

*Succinea retusa* (Lea)

*Cochlicopa lubrica* (Mull.)

Many representatives of each of these species were exposed individually, kept under observation during exposure, and later killed and examined for any stages of intra-molluscan development. In no instance were any stages of development of *F. hepatica* observed in these snails. Later in the investigation over 25 tanks of snails were exposed to mass infections of miracidia, these infections being repeated at intervals of a few days, thus permitting ample opportunity for infestation of the intermediate host. Abnormal mortality of the molluscs was never noticed after exposure. Those that died were examined immediately, but did not yield any stages of *F. hepatica*.

On exposure to miracidia, all species were readily attacked, those of non-Lymnaea groups being attacked almost as readily as those of the Lymnaea types. Although the attack was deliberate in many cases, it was also acci-

dental in others, for on many occasions miracidia were observed to swim by the snails without attacking them. This occurrence, however, was also noted when a proven vector, *Gyraulus ferruginea* Haldeman, was exposed to their attack. The miracidia would definitely attack, but they would also swim around in close proximity without becoming attracted or attempting attachment to the tissues of the snail. During the periods of exposure, only one miracidium was definitely seen to enter the tissues of the mollusc.

Mattes (5) has shown that there is no evidence of chemotaxis in the finding of the intermediate host by the miracidia. He observed that they appear to reject hard bodies such as stones, but they repeatedly attack soft-bodied animals. He considers that the finding of an intermediary is a random process.

In many cases miracidia, on introduction of a mollusc to their dish, would attach themselves to any exposed portion of it. At times they would detach immediately and at others would remain attached for 10 min., attempting penetration of the host's tissue, but never succeeding. On several occasions, after attacking for some little time, a paralysis of the organism would appear to occur, the miracidium finally dropping away from its point of attachment as if exhausted. It is possible that this paralysis may be the result of some defence mechanism on behalf of the mollusc. However, the snails appeared unaware of any external irritation during periods of attack.

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# LACUSTRINE INVESTIGATIONS IN THE GASPÉ PENINSULA<sup>1</sup>

BY A. J. M. HONEYMAN<sup>2</sup>

## Abstract

A study of the temperature variations in Ross Lake indicates that it is a typical "second order" lake. The seasonal variations are explicable in terms of the changes in air temperature and the resulting effects on temperature and density of the water. Thermal stratification is clearly indicated. Seasonal variations in the dissolved oxygen content of the water are largely dependent on temperature changes. The variations in acidity, alkalinity, and hydrogen ion concentration are dependent chiefly on temperature changes affecting solubility of carbon dioxide, and on the limy nature of the lake bottom. Analysis of the oxygen distribution indicates a eutrophic condition, but there is as yet insufficient quantitative information about the biological conditions to warrant definite conclusions.

## Introduction

In the Gaspé peninsula, about 15 miles from the village of Gaspé, are eight small lakes that are used by the Hatcheries Branch of the Department of Fisheries as natural rearing waters for trout brood stock. The general features of these lakes are discussed in a paper by Taylor<sup>3</sup> and Lindsay (10). The lakes are similar in some respects, but diversified as to size, depth, and type of bottom. They are, with the exception of Grand Etang Lake, within a radius of three miles in a region sufficiently mountainous and inaccessible to be visited only by local woodsmen. They vary in size from Fourth Lake, with a length of about 1.6 km. and a breadth of 800 m., to the pond-like Trail Lake, whose length and breadth each approximate 180 m. The depth varies from slightly over 30 m. in Grand Etang to between 3 and 4 m. in Trail Lake. The entire bottoms of the smaller lakes and parts of those in the larger ones are formed of limestone or a deposit of soft, almost pure calcium carbonate. There is very little of this deposit in Fourth Lake. The importance of such deposits in the economy of lake productivity is worthy of investigation. These lakes afford an opportunity to investigate problems of fertility and productivity, which may be of general application.

For the past three years an intensive study has been made of Ross Lake with reference to topography, water temperatures, dissolved oxygen content, acidity, alkalinity, and hydrogen ion concentration. Similar but less extensive studies have been made on several other lakes. A study of biological factors, including the use of algal photosynthesis as a measure of productivity, is now being undertaken.

## Methods

### Temperature

Temperatures were obtained with a Richter and Weise reversing thermometer at one point within the 21-m. contour. In winter they were taken through a hole in the ice, in summer, from a boat.

<sup>1</sup> *Manuscript received in original form February 23, 1939, and as revised, July 31, 1939. Contribution from the Department of Mines and Fisheries, Quebec. Presented in briefer form to the Limnological Society of America at Richmond, Virginia, on December 28, 1938.*

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### *Dissolved Oxygen*

The method used in the determination of dissolved oxygen is a modification of the standard Winkler technique, the chemicals used and the reactions involved being the same. This method, described by Nicloux (8), was found very suitable to the conditions of our work. The advantages of the technique are that smaller samples of water are required and that no chemicals need be added at the time of collection. Only about 25 ml. of water is used for an analysis. The analysis itself is very rapidly carried out, owing to the fact that small quantities of chemicals are used. Samples analyzed at the time of collection and others that had been kept in bottles under water and packed in ice, gave identical results. The water samples were collected with a Richter and Weise reversing water bottle and run into glass-stoppered bottles that were stored in special ice-cooled metal containers. The analyses were made in a laboratory on shore.

### *Acidity and Alkalinity*

Free carbon dioxide content and alkalinity were determined by neutralization of the sample in a Nessler tube with standard sodium hydroxide and sulphuric acid solutions respectively, phenolphthalein being the indicator. The technique is that of the American Public Health Association (1, p. 32 *et seq.*). The carbon dioxide determinations are expressed as parts per million of carbon dioxide and the alkalinity as parts per million of calcium carbonate.

### *Hydrogen Ion Concentration*

These determinations were made colorimetrically by use of the indicators brom thymol blue, phenol red, chlorphenol red, and metacresol purple.

## **Ross Lake Topography**

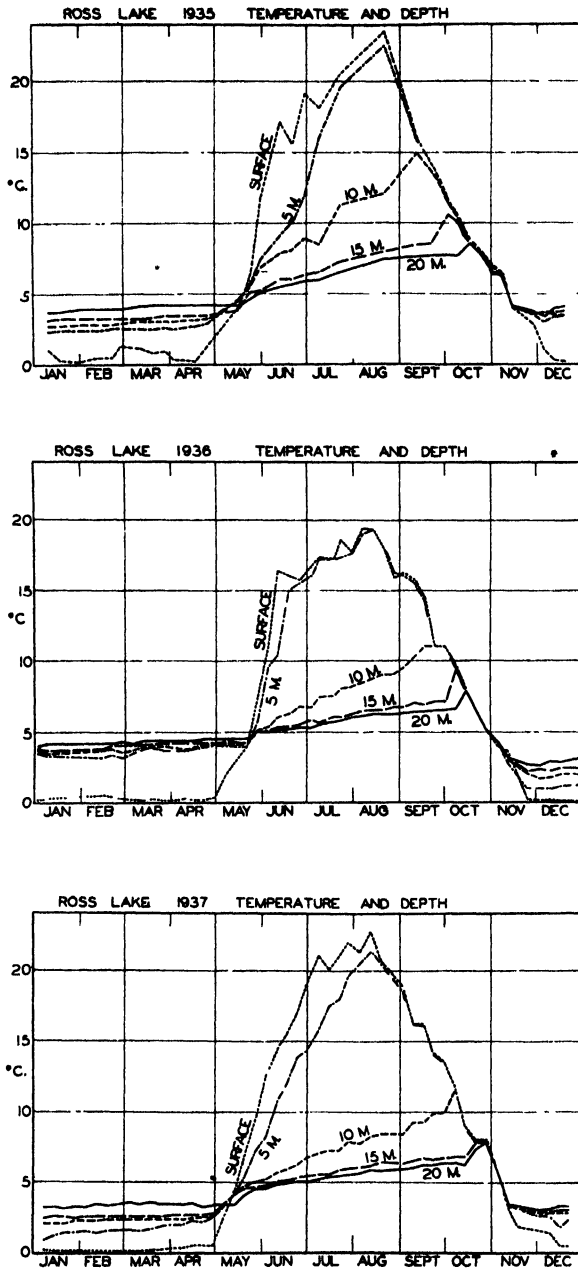
Ross Lake is situated in the county of Gaspé South, township of Baillargeon, near the eastern extremity of the Gaspé peninsula and about 15 miles from the village of Gaspé. It is on top of a high ridge, with the surface at an altitude of 164 m., a situation that provides a comparatively small drainage basin and no inlet brooks. Its outlet is small and empties into the St. John River. The drainage area is only about three times the area of the lake itself, which is fairly regular in shape and measures 710 m. in length and 610 m. in breadth. Depth contours have been mapped and were derived from an extensive series of soundings taken at 100-ft. intervals over the surface when it was covered with ice.

There are two deep areas in the lake, one of 23 m. and another of 20 m. A very large percentage of the lake is over 9 m. in depth. The only shallow areas of any extent are in the vicinity of the discharge. More than half the drainage area is covered by a poor growth of young poplar.

## **Temperature Conditions**

The temperature data on conditions in this lake are plotted over yearly periods in Figs. 1, 2, and 3. These three graphs show the seasonal variations

in temperature for the complete years 1935, 1936, and 1937, the measurements being made at weekly intervals, and at depths of 20, 15, 10, and 5 m. and the surface. Examination and comparison of these graphs disclose some interest-



FIGS. 1 - 3. Curves for Ross Lake, 1935 (top), 1936 (centre), and 1937 (bottom), showing the seasonal variation of temperature with depth. Readings taken at weekly intervals all from one station.

ing facts. Their similarities may be summarized as follows. The spring overturn occurs about May 15, and the period of complete mixing is confined to about a week. Then thermal stratification sets in and becomes most pronounced in mid-August, when a difference as great as 8° C. exists between the water at 5 and 10 m. The autumn overturn occurs about October 15; but the stratification that follows is evidently a slower process, as indicated by the curves for different levels. The temperatures do not begin to diverge for about a month after all levels reach the same temperature. This is to be expected, because once the ice cover is present conditions are relatively static, and the stratification is brought about only by the effect of gravity. When the winter stratification is complete, the temperature varies in a regular manner from 0.1° C., at the surface (*i.e.*, the point just below the ice) to approximately 4° C., the temperature of maximum density, at the bottom. Evidently when disturbing external forces are excluded by the ice the water layers are distributed according to their density. During the ice-free months the temperature—depth relation is exactly reversed, though the density relation holds, the layers of water farthest removed from the temperature of maximum density being nearest the surface.

Graphs for all years show also that the maximum temperature for any depth is reached later in the season as the depth increases. This is to be expected since the only source of heat for the lake is the warm air above it, and the upper layers of water must be heated before those below can be affected. This is a slow process and is unlikely to be aided to any extent by currents, due to the resistance of the stratified water. The October circulation period is made possible by the disappearance at this time of thermal stratification, and it is shown that this homothermous condition is brought about not by any considerable warming of the lower water levels but almost entirely by the cooling of the surface layers to a temperature equal to that of the bottom layers.

No precise study of the thermocline can be made from the data, since the depth intervals (5 m.) are too great, but there are certain points of interest. In 1935 one definite thermocline emerges in July, at a depth between 5 and 10 m. By September its position has sunk to between 10 and 15 m. In 1936 on the other hand, the thermocline remains established between 5 and 10 m. from June until early September. In 1937 the typical depression of the thermocline with the advancing season is shown more clearly than in 1936.

As would be expected, the maximum temperature of the epilimnion shows some variation from year to year, but the time is always between August 7 and 21.

Some observations will now be made on the intermediate levels of 5, 10, and 15 m. In 1936, May 21 is the only date (as judged from temperatures) when there is practically complete mixing of the water of the lake. This is the focal point of the spring overturn. The temperatures at 20, 15, 10, and 5 m. and at the surface on this date are respectively 4.5, 4.2, 4.1, 4.1, and

4.0° C. On May 17, the previous date on which readings were taken, the surface water was at 2° C. while on May 28, it had warmed to 7.4° C. The fall overturn is a slower process than the spring circulation, and the whole body of water in the lake remains homothermous from October 15 to November 12, though during this interval it cools gradually from 8° to 3° C. A few days after November 12 the ice cover appears, and a rapid cooling of the surface water to its winter level takes place.

Measurements from these intermediate levels show also the gradually changing seasonal heat distribution in the lake. At the beginning of June 1936 the water begins to warm from the surface downward, the epilimnion region reaching a depth of 10 m. on September 10. This region maintains a temperature of 10° C. or higher until October 1, when further cooling and mixing equalizes the temperature of the whole body of the lake. These observations apply also to the data obtained in 1937.

The lake may then be classified on the basis of the above temperature relations, as one of the "second order" in the scheme developed by Forel and Whipple.

### Dissolved Oxygen

Measurements of dissolved oxygen in Ross Lake are available for the complete years 1936 and 1937. The data have been calculated in parts per million and percentage saturation. The results for the surface and 20 m. depth are given in Figs. 4 to 7.

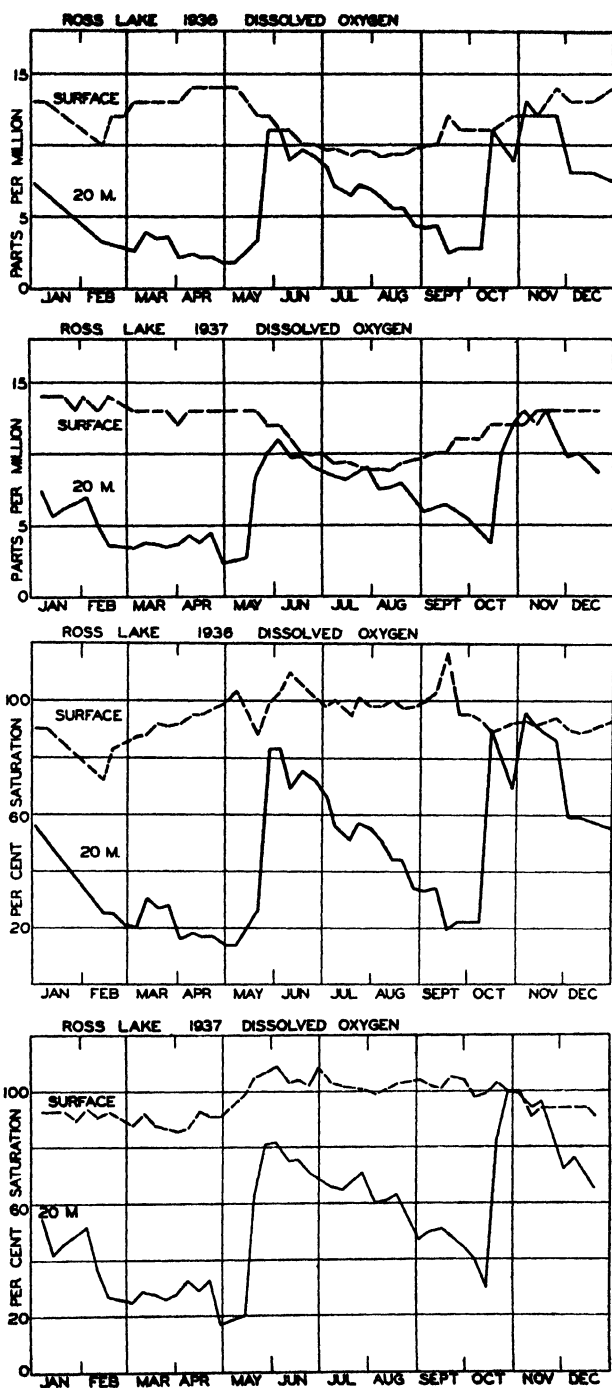
Examination of the graph showing the seasonal variation in absolute dissolved oxygen content for 1936 (Fig. 4) reveals the following significant points.

The oxygen content of the surface water is high (12 to 14 p.p.m.) when the ice cover is present, that is, from January to May and from October to December inclusive. This is due chiefly to the fact that the temperature is approximately 0° C., at which point the solubility of the gas is at a maximum.

The drop in oxygen content of the surface water from June to August is due to the higher water temperature and consequent slighter solubility of the gas. The initial drop results from the water in the lake being mixed at the spring overturn, the surface water losing oxygen to the lower levels.

The same graph shows how the oxygen content of the whole lake becomes uniform within the short period of a week at the end of May as a result of the spring overturn, though this process probably does not greatly increase the absolute quantity of oxygen in the lake. The rapidity with which this uniformity develops is a result of the rapid disappearance of the ice after it begins to melt. Once it has broken, the ice disappears within a day, and the mixing process begins immediately.

Another point shown by the data is that in July and August the oxygen content is not greatest at the surface, as one might expect, the maximum being at 10 m. It is supposed that the higher temperature of the surface water at this time reduces solubility, in spite of the fact that more oxygen is available



FIGS. 4-7. FIGS. 4 AND 5 (TOP). Ross Lake 1936 and 1937. Seasonal variation in dissolved oxygen content shown for surface and 20 m. depth. Measurements all made at same station. FIGS. 6 AND 7 (BOTTOM). Ross Lake 1936 and 1937. Dissolved oxygen expressed as a percentage of the maximum amount soluble in distilled water at the same temperature.

at the surface than at 10 m. For the same period it is seen that the decline in oxygen content above 10 m. involves no decline in percentage saturation. It would be desirable to study the presence in these regions of algal growths, as a probable factor in this situation.

Water at the 20 m. level after June 1 gradually loses oxygen (a) because it is too far from the source of supply to receive any by diffusion, and being below the thermocline it is unlikely that water currents will supply it to any extent, (b) because of its slowly rising temperature and (c) probably because of decomposition activity, though the extent of such processes cannot yet be estimated in this lake.

At the autumn overturn the oxygen content again becomes uniform throughout. The drop in oxygen content of the lower levels with the advent of the ice cover in November is due to diffusion of gas to the colder surface layers.

A curve relating percentage saturation of oxygen and depth for 1936 is given in Fig. 6. The data have been calculated from the measurements of C. J. J. Fox, as tabulated by G. C. and M. C. Whipple. It is noted that this temperature adjustment has practically no effect on the shape of the 20 m. curve, its outline being the same as that for absolute oxygen content at the same level. A noticeable change has been produced in the surface water curve. The decrease in oxygen content during the summer months is now not shown because, as was noted above, this decrease was due to smaller absolute solubility of the gas in the warmer water. The percentage of oxygen dissolved is only slightly higher during the summer season than during the winter, the difference being due probably to photosynthetic activity.

In regard to the lower and upper limits of oxygen concentration the following points may be noted. The lowest concentrations are found in bottom water during the stagnation periods and vary from 2 to 4 p.p.m., the lowest value obtained in the two years being 1.8 p.p.m. The highest values are found in surface waters during the winter months and frequently reach 14 p.p.m.

Values for percentage saturation are as low as 13.7 at a depth of 20 m. in March and April before the ice melts. Readings for surface water of over 100% saturation are frequently found, though no large masses of algae or other aquatic plants are present in this lake. Comparison of the oxygen curves for 1937 with those of 1936 indicates a remarkable similarity. The spring and fall overturns each come a few days later in 1937. The percentage saturation curves for 1937 (Fig. 7) are likewise similar to those of 1936.

In regard to the oxygen content at depths intermediate between 20 m. and the surface, from January to June, 1936, the amount of oxygen decreases in a regular manner from surface to bottom, though after the spring circulation there is a considerable increase at the lower levels. At the overturn the whole body of water is almost saturated with oxygen. In July, however, a different situation develops, and, when the temperature of the epilimnion rises, the amount of gas dissolved in it decreases to about 9.5 p.p.m. The intermediate

region (about 10 m.) has the greatest concentration of oxygen at this time. This situation does not last long, however, for by September the epilimnion has extended downward to include the 10 m. level. In November the autumn circulation brings about an even, vertical distribution of oxygen. The data for 1937 confirm these observations.

### Further Analysis of Physical and Chemical Conditions

A summary of the chief physical and chemical characteristics of Ross Lake is found in Table I. The small area and volume make its comparison with other lakes on an equal basis difficult. The volume of the hydrographic epilimnion (0 to 10 m.) is about three times that of the hypolimnion. Such a ratio is considered to be an indication of a eutrophic condition. The annual heat budget, calculated by the method of Birge, is lower than that usually found, probably because of the small volume and area.

TABLE I  
ROSS LAKE. SUMMARY OF PHYSICAL AND CHEMICAL CONDITIONS

Date	Aug. 6, 1936		
Latitude	49		
Altitude (m.)	164	<i>Oxygen amounts</i>	
Area (km. <sup>2</sup> )	0.26	O <sub>2</sub> E. (cc./l.)	6.8
Length (km.)	0.71	Total O <sub>2</sub> E. (cc.)	1208 × 10 <sup>7</sup>
Breadth (km.)	0.61	O <sub>2</sub> H. (cc./l.)	5.2
Mean depth (m.)	9.1	Total O <sub>2</sub> H. (cc.)	321 × 10 <sup>7</sup>
Max. depth (m.)	23	O <sub>2</sub> H./O <sub>2</sub> E.	0.27
Volume (m. <sup>3</sup> )	2394 × 10 <sup>3</sup>	<i>Oxygen deficits</i>	
Vol. E. (0-10 m.)	1776 × 10 <sup>3</sup>	ΔE (cc./l.)	0.2
Vol. H. (10 m.-bottom)	618 × 10 <sup>3</sup>	ΔH (cc./l.)	3.5
Vol. H./Vol. E.	0.35	ΔH + E (cc./l.)	1.05
Annual heat budget	11,100	<i>Oxygen capacity</i>	
(gm. cal./cm. <sup>2</sup> )		O <sub>2</sub> A (cc./cm. <sup>2</sup> )	0.97

An analysis of the oxygen conditions has been made by the methods of Thienemann (11). The calculations were made for Aug. 6, 1936, a date representative of midsummer conditions. The ratio O<sub>2</sub>H/O<sub>2</sub>E of 0.27 is typical of that found in eutrophic lakes (9). In calculating the oxygen deficits the oxygen solubility values used are those of Whipple and Whipple. The deficit for the epilimnion (ΔE) is slight, 0.2 cc./l. indicating that this layer is practically saturated. In fact, on some days negative deficits are found, indicating supersaturation in this layer. For the hypolimnion the oxygen deficit is considerably greater, amounting to 3.5, though not high in comparison with many of the eutrophic lakes listed by Rawson. The deficit for the whole lake, 1.05, is derived from ΔE and ΔH by considering the respective volumes of E and H, and as a result is closer to the ΔE value. These statements may be confirmed in a general way by examining the saturation curves (Figs. 6 and 7). The analysis of oxygen conditions indicates a

eutrophic condition, but more definite conclusions cannot be drawn until further biological and chemical information is available.

### Acidity and Alkalinity

The data with reference to these factors are plotted on the same graphs (Fig. 8 for 1936 and Fig. 9 for 1937), the carbon dioxide being represented above the zero line and the alkalinity figures below it. Only data for measurements at the surface and 20 m. depth are shown on the graph.

At a depth of 20 m. the carbon dioxide content of the water increases gradually from January to the middle of May, when the spring overturn takes place. It then drops to almost zero, begins to rise during the summer, and falls again with the autumn overturn.

The surface water shows less variation throughout the year. It is acid from December to May and on the alkaline side during the summer months. The conditions are similar in 1936 and 1937. It may be said that the surface waters of Ross Lake contain little or no free carbon dioxide. This may be explained by the fact that the water is constantly agitated and the dissolved carbon dioxide escapes by diffusion into the air, and by the buffering effect of the carbonates present.

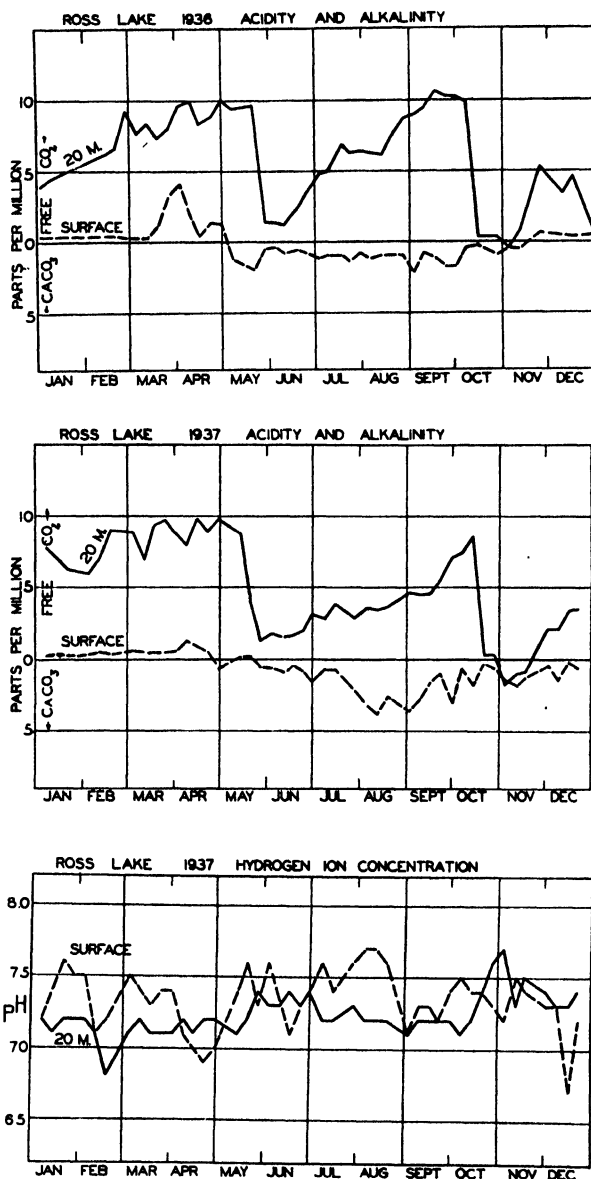
From January to April 1936, the carbon dioxide content of the lake increases gradually from surface to bottom. In May the surface and 5 m. levels lose all their carbon dioxide and become alkaline. This condition is maintained until September, when the 15 m. level also becomes alkaline. For a brief period at the autumn overturn (November 5) the whole body of water is alkaline in reaction.

It is noteworthy that at two periods of the year the lake loses its entire content of carbon dioxide, which is essential for organic productivity. In addition to this reduction at the circulation period, the activities of certain marl-forming algae (*Chara*) and pondweeds (*Potamogeton*) precipitate carbon dioxide as insoluble carbonate. Any photosynthetic activity has the same effect, but these particular genera are found in great quantities in Ross Lake; in fact, they compose almost the entire rooted standing plant growth. Their activity is indicated by the large beds of marl on the bottom of the lake. These marl deposits are formed chiefly in the epilimnionic region. In some other lakes of the vicinity, shallow enough to be homothermous at all times, the deposits cover the entire bottoms. This distribution is in accord with the observations of Kindle (6), who points out that all the factors tending to produce marl deposits are most active in the warmer surface layers of the lakes.

### Hydrogen Ion Concentration

Data on pH are available for the period August to December 1936 and the year 1937, and the results of the surface and 20 m. depth determinations of the latter year are plotted in Fig. 10. The maximum pH range in Ross Lake is from 6.8 to 8.1.

A comparison of the free carbon dioxide graph for 1937 (Fig. 9) and the pH graph for 1937 (Fig. 10) shows that in spite of great irregularity in individual measurements, there is a considerable correspondence between the seasonal changes in the two cases. For instance, a fall in carbon dioxide content at 20 m. in mid-May corresponds to a rise (more alkaline) in pH at that time.



FIGS. 8 - 10. FIGS. 8 AND 9 (TOP AND CENTRE). Ross Lake 1936 and 1937. Free carbon dioxide indicating acidity is shown in the upper part of the graph while the lower part shows alkalinity expressed in parts per million of calcium carbonate. FIG 10 (BOTTOM). Ross Lake 1937. Showing the seasonal variation in pH at the surface and 20 m. depth.

Increasing alkalinity of the surface water in July and August corresponds to an increase in pH at this time. Similar relations are observable at other seasons.

On the whole the seasonal variations in pH are slight. It is suggested that this may be due to the buffer action of the monocarbonates present. As mentioned above, large quantities of calcium carbonate are present both as bottom deposits and as constituents of, and incrustations on, water plants such as *Chara* and various Potamogetons. Any increase of free carbon dioxide or carbonic acid content in the presence of calcium carbonate is immediately neutralized, and little increase in pH occurs. Likewise, removal of carbon dioxide by photosynthetic activity or by agitation of the water results in the decomposition of bicarbonate into carbonate and carbonic acid, thus increasing the acidity.

### Summary of Physical Conditions

The physical conditions in Ross Lake are in most respects favourable to aquatic life. In comparison with the size of the lake, there is a large body of deep water, a considerable part of which is cold owing to the presence of a definite thermocline. However, the large proportion of deep water results in a corresponding lack of shallow areas adequate for organic growth. Another marked deficiency is in the absence of inlets to the lake, because of its position on a height of land. The drainage basin itself is covered by a scanty growth of young poplar and there is very little top soil over the rocks. The temperature, typical of such lakes, is favourable to fish life.

The dissolved oxygen content shows the expected seasonal variations. Though authorities differ on the minimum and optimum oxygen requirements of fish, it is unlikely that the supply here becomes inadequate except at the deep levels during the winter months. However, a situation unfavourable to fish life may exist in the coincidence of low oxygen periods and time of acid pH.

The lack of carbon dioxide at certain seasons is shown by the data and curves. The pH variations follow in general the carbon dioxide cycle, but the seasonal changes are less well defined and in any case are confined to a range of pH 6.8 to 8.1.

### Biological Conditions

In 1887, Forbes (5) spoke of the lake as a closed community comparatively slightly affected by events outside it, and in 1935 Welch (12) stated that "in a lake with a small inflow and outflow of water, relatively small slow additions to the food supply are made from the outside." Klugh (7) and other writers attached importance to the quantity of rooted aquatic vegetation as an index of productivity of lakes. These viewpoints have special application to the lakes herein studied. Plant life in all these lakes is scarce. The only plant that can be described as abundant and well established is *Chara*. The rest of the plants are seen as scattered specimens that have made little progress in occupying the large vacant areas of lake bottom. There are two

possible explanations of such a paucity of plant life. That of the absence of shallow areas near shore and the regularity of the shore line applies to Grand Etang, McLaren, and Ross Lakes. The other and related explanation, that of the absence of sufficient food material to support any considerable plant population, is generally applicable. The lakes are practically devoid of soil bottom, the bottom consisting of limestone or of soft marl deposit often a foot or more thick. Certain conditions tend to restrict the bottom deposits to this marl. It is strangely characteristic of these lakes that they are situated on high elevations and consequently possess comparatively small drainage basins. This reduces the run-off of organic material into the water. In any case, the forest cover about Ross and Fourth Lakes is very light, and the soil only slightly covers the rocky ground. Also, owing to the small size of the lakes the effect of wind and wave action in eroding the shores is slight. It should be noted as well that in common with all other lakes at this latitude, organic activity on the land surrounding the water is in an even more static condition than in the water itself for more than five months of the year. The apparent inability of these lakes to support a large fish population for more than a few years is an indirect indication of the deficiency of available organic material.

The following genera of water plants are generally though sparsely distributed throughout the lakes: *Chara*, *Potamogeton*, *Myriophyllum*, *Nymphaea*, *Sparganium*, *Eriocaulon*, *Fontinalis*, *Equisetum*, *Utricularia*, and *Oedogonium*. Of these, the lime-consuming plant *Chara* and the *Potamogetons*, on which quantities of lime are deposited, are the dominant species.

A more quantitative study of the biological factors will be undertaken before more definite estimation of their effects is made.

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## THE DETECTION OF ABNORMAL COW'S MILK BY MICROSCOPIC METHODS<sup>1</sup>

BY SEYMOUR HADWEN<sup>2</sup> AND RONALD GWATKIN<sup>3</sup>

### Abstract

A large number of milk samples from dairy cows have been studied microscopically and culturally, the object being to estimate the numbers of body cells and leucocytes present.

The Breed and sediment counts are compared. The latter is preferred because it furnishes more information than mere enumeration of the cells. In a sediment smear a complete picture of conditions in the udder may be visualized, including the various cellular responses to the organisms causing mastitis. Estimates of the numbers of cells present are sufficient for a diagnosis when accompanied by the information gained in examining the smear.

Differential counts were made for special purposes only, such as to define normality in milk. In defining normal milk, a standard was set which did not tolerate the presence of micro-organisms or polymorphonuclear leucocytes in the samples. In a herd of 60 cows, milk from 9 young cows met this standard. Diphtheroids occurred in 70% of the cows. In comparison with the clean cows, the diphtheroid carriers had larger numbers of polymorphonuclear leucocytes.

In staphylococcal mastitis the leucocytes are often very numerous, and this may persist for months. Staphylococcal infections cause a great influx of large, ring-shaped polymorphonuclear leucocytes. The cocci can generally be found on the smears. Sometimes, when leucocytes are scarce, cocci occur in very large numbers.

In streptococcal mastitis the mononuclear leucocytes are numerous. Loose, irregular clumping is commonly seen, and the polymorphonuclear leucocytes often clump separately. Leucocytes are not as numerous as in staphylococcal cases, but the percentage of the mononuclear leucocytes is higher. Tables showing the increase and decrease of the leucocytes indicate that when they are numerous the infective organisms may be scarce or absent, the reverse being also true. Streptococcal mastitis is sometimes a difficult disease to diagnose microscopically, on account of scarcity of organisms. Various ways of finding the cocci are discussed.

The diagnosis of *B. coli* and *Corynebacterium pyogenes* mastitis is described. Both these organisms may cause severe lesions, and consequently the smears reveal extensive degenerative changes in the leucocytes. The organisms are present in large numbers. *B. coli* and *C. pyogenes* infections produce a different leucocytic picture under the microscope than do pyogenic cocci. The effect of these infections is generally more destructive.

A study of phagocytosis in diseases of the udder furnishes valuable aid in determining the degree of resistance to infection on the part of the host.

Red blood corpuscles in milk may be unaccompanied by any signs of infection. Chromatin-staining granules in milk are caused by degenerative changes in the polymorphonuclear cells. Calcium calculi are found both in the tissues and in the milk. A study of over 20 samples of colostrum is recorded.

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## Introduction

The present study relates principally to the different kinds of cells and organisms encountered in milk, and their functions and significance. An examination was made of the milk of 1700 cows, which figure includes the retesting of some of the herds under observation. The results of the microscopic examination were compared with cultural tests carried out simultaneously. These results, together with the percentages of the various organisms encountered, were published in 1937 (5) and will be only briefly referred to here.

Sections of udders have been cut, for the purpose of comparing the cells found in the ducts and alveoli of the udder and those that occur in the milk, and this comparison showed that the cells appear to be identical. Pathological changes accompanying disease have been studied. The above studies naturally bear on the diagnosis of mastitis through microscopic examination. Our principal aim has been to find a method of diagnosis that could be used by practising veterinarians.

## The Significance of Leucocytes in Milk

The three volumes compiled by E. Munch-Petersen in Australia have given us much valuable information on mastitis (11). In the words of the late Dr. J. A. Gilruth: "It affords us the most complete résumé on the work accomplished up to the present time".

The following quotations, drawn from the work of investigators in four different countries, do not necessarily represent the opinions held in those countries, but they are the results of well-considered and extensive investigations.

Minett, Stableforth, and Edwards (9) state: "The microscopic examination of centrifuged milk sediment for streptococci is much less reliable than the cultural method. In individual cases, or when small numbers of samples have to be examined, the method may give useful results, because if these are positive, cultural work may be rendered unnecessary."

"The microscopic examination of cream smears to obtain information as to the type and number of cells present is a simple and easily applied method which may serve to direct attention to abnormalities in the gland. As would be expected, however, there are a number of border-line cases in which a correct interpretation is difficult, and the method gives no definite clue to the species of bacteria responsible."

The quotations show that these authors do not favour the examination of either milk sediment or cream under the microscope as a means of diagnosing mastitis. They give figures showing that they were unable to detect streptococci in 47.5% of the cases examined.

Our figures published elsewhere (5) show that we were able to detect 75% of streptococcic cases, and 17% that showed numbers of leucocytes but no streptococci. A further 7.5% showed nothing. These cases were reported

before we had completed our field studies. If other forms of mastitis were included with the streptococcic cases, the results would be even better. It should be pointed out that Minett *et al.* did not mention the types of cells they encountered, while we have found this information valuable as an indication of various responses to infection. The stains they used would preclude to a considerable extent the determination of certain types of cells. Also, examination often reveals whether the leucocytes are combating infection by their phagocytic action, or whether the infection is gaining, as indicated by few leucocytes and free organisms in the milk.

Bourgeois (2) has devoted much time to the study of leucocytes in milk. He advises heating the milk to 70° C. before centrifuging. Giemsa's stain is used. Referring to polymorphonuclear cells, Bourgeois quotes Rulot and Marchal's statement that these cells are able to dissolve fibrin and that there is an extra- and intracellular action on lipase. He believes that no analogous facts have been noted in milk. We have shown that the polymorphonuclear cells commonly ingest mucinous material, and also that the macrophage cells are sometimes gorged with fat globules and assist in getting rid of red corpuscles. Bourgeois defines colostrum as a "réliquat de phagocytose d'un lait antérieurement produit, ainsi que l'ont défini—C. Porcher et L. Panisset, offre ces particularités au suprême degré." Bourgeois concludes by quoting Porcher, with whom he agrees, that "la mamelle saine est l'exception alors qu'au contraire la mamelle infectée est presque la règle".

Hopkirk (6) outlines his method of cream examination as a practical way of diagnosing mastitis. "Its general idea is the grouping of cows in a herd for milking purposes on the basis of the number of leucocytes present in the milk, as disclosed by careful microscopical examination, carried out at monthly intervals". Samples of the "fore" milk are drawn, or these may be composite samples of the whole milking. Cream smears are made with a 2-mm. loop, from an area 1.0 to 1.2 cm. in diameter. The smears are fixed lightly with heat, put through xylol and alcohol, and stained with methylene blue.

It is evident that Hopkirk's ideas and our own are similar regarding leucocytes as indicators of disease, but they differ in method of application. Our object has been to separate the cells in the milk from the cream, without injuring them or disturbing their arrangement, so that the various types of cells in association with the infective organisms may be differentiated. We have been unable to obtain uniformity in our preparations when much fat is present, or when xylol is used to remove it. Methylene blue is not a satisfactory stain, any more than it would be for a blood smear. A quick-acting blood stain, such as Hastings', gives preparations in which the various types of leucocytes may be identified. Concerning differential counts, we agree with Hopkirk that for general purposes of grouping cows in the herds, it is sufficient to indicate the approximate numbers of leucocytes. But with experience, much more than that can be determined, such as the differences that can be detected microscopically between the diseases and other abnormal conditions affecting the udder.

## Methods Used in the Preparation and Examination of Samples

Before the milk sample was taken, the teats were washed with sodium hypochlorite solution, separate cloths being used for each cow, and the first few streams were discarded. The milk was cooled with ice and examined as soon as possible. The method of making smears is compared with the Breed (3) method in the following section. In staining the smears with Hastings' stain, we have found it advisable to overstain rather than understain. If the stain does precipitate a little it can easily be removed by allowing a few drops of alcohol to run over the slides. Deep staining is necessary to show mucinous material within the polymorphonuclear leucocytes. The coloration of the smears varies considerably between normal and pathological milk. Some of the smears tend to wash off when the stain is removed. In such cases it has been found best to dry the films without washing. After drying, the excess stain is removed with alcohol.

In classifying and enumerating the leucocytes, each smear was crossed once, and when the far edge was reached it was followed around to the starting point. In this way both the middle and edge of the film were examined. This is advisable because, in the process of drying, the leucocytes tend to congregate in little pools and the larger cells run to the edges. When it was desired to make sure that no polymorphonuclears were present, a search was made along the edges. It has been noted repeatedly that cells filled with cocci tend to gravitate to the edges. The large epithelial scales apparently do not follow this rule and may be found anywhere on the smear. In films so thickly covered with leucocytes that they touch one another, these distributions are not as noticeable.

Plate cultures of the milk samples were made in the usual manner with blood agar plates. In order to obtain unbiased results, the cultural and microscopic examinations were conducted separately. Stained smears were diagnosed by one author (S. H.), and later these results were compared with the cultural data obtained by the other author (R. G.).

### A COMPARISON BETWEEN THE BREED AND SEDIMENT METHODS OF COUNTING LEUCOCYTES IN MILK

In the Breed method (3), 10 cc. of whole milk is shaken and 0.01 cc. spread over 1 sq. cm. on a slide. Alcohol and xylol are used to fix the smear and to remove the fat. The smears are made in duplicate. A methylene blue stain is used.

In the sediment method, 10 cc. of milk is centrifuged for 10 min. at 2500 to 3000 r.p.m. The cream is wiped out of the neck of the sample bottles with cotton. The sediment removed by a platinum loop holding about 0.005 cc. is spread over a surface of 2 sq. cm. on a slide. The smears are air-dried and stained with Hastings' blood stain. The field covered by the microscope is 0.16 mm., as advocated by Breed.

The results of a comparison between these two methods on the milk of eight cows are given in Table I. The figures show a similarity between the sediment and Breed counts in the low numbers, but otherwise are generally much higher by the former method.

TABLE I  
COMPARISON OF SEDIMENT AND BREED COUNTS

Cow	Quarter	Leucocytes per 100 fields		Blood agar plate cultures
		Sediment	Breed	
1	RF	9	9	Clean
	RH	6	6	2 colonies bacilli
	LF	24	19	Diphtheroids
	LH	9	4	1 diphtheroid
2	RF	20	18	1 colony cocci
	RH	15	17	Clean
	LF	3	17	2 colonies
	LH	7	26	Diphtheroids numerous
3	RF	26	11	3 colonies bacilli
	RH	45	10	Diphtheroids
	LF	44	9	Diphtheroids
	LH	38	13	Diphtheroids
4 (Newly freshened)	RF	17	15	13 colonies coarse cocci
	RH	29	39	10 large cocci
	LF	27	15	5 large cocci
	LH	37	13	4 large cocci
5	RF	22	12	1 colony diphtheroids
	RH	23	11	Diphtheroids
	LF	2610	372	36 colonies staphylococci, 2 diph.
	LH	29	91	No colonies
6	RF	40	28	1 colony
	RH	5	6	1 colony bacilli
	LF	2330	394	5 colonies staphylococci
	LH	29	26	5 colonies bacilli
7 (Newly freshened)	RF	109	13	1 colony contaminants
	RH	171	19	No colonies
	LF	455	136	46 colonies staphylococci
	LH	13	16	A few diphtheroids
8 (Newly freshened)	RF	88	102	9 colonies cocci
	RH	21	9	8 colonies large cocci
	LF	1130	57	30 colonies staphylococci
	LH	15	10	No colonies

*Cows 1, 2, and 3 show infection with diphtheroids. Prior to the last test they had shown no organisms and very few leucocytes.*

Except in special instances, such as the above and the attempt to define normal milk, we have not made complete leucocyte counts, but simply estimates of the numbers. In pathological milk it is often difficult to distinguish many of the leucocytes sufficiently to make a reliable count, although in

numerous samples the cells stain as perfectly as they do in blood. Pathological samples in which the leucocytes are scarce occur fairly often in mastitis. This scarcity is owing to a lack of resistance on the part of the cows, which would be passed as clean if the leucocyte count was relied on alone. With the Breed method it was impossible to identify as high a percentage of cells as with ours, because of inferior staining. Breed counts alone do not furnish a definite diagnosis for mastitis, but merely indicate the number of leucocytes present.

The principal reason for adopting the sediment method is because it is a simple and direct way of examining milk. If the sediment samples are taken carefully and examined quickly, the only organisms present are those that have come directly from the udder. The arrangement of the leucocytes may be informative. For example, clumping indicates a defensive reaction; numerous lining cells indicate a destructive infection, and the causative organisms will probably be seen; phagocytic activity of the leucocytes may indicate whether a case of mastitis is in the acute or chronic phase. Other abnormalities in milk may be seen, such as blood, mucin, calcium calculi, and various micro-organisms. The concentration of the leucocytes in the sediment is sufficient in most instances to give a clear picture of the reactions that are taking place.

### **An Attempt to Establish the Normal in Cows' Milk**

Munch-Petersen (11), in a complete survey of the literature on bovine mastitis, draws the following conclusion: "What constitutes normality so far as the bovine udder and mastitis are concerned? Nowhere in the literature perused in preparing this summary has the writer found adequate work on this fundamental important point." "The question whether the udder is normally sterile or is inhabited by micro-organisms -- cannot be said to have been definitely settled."

In view of the divergence of opinion among various workers, it is desirable to state as clearly as possible what degree of cleanliness and freedom from infection has been sought in this attempt to define normal milk. Regardless of the implication, and disregarding the previous figures we have published, it was decided to re-examine the smears from the cleanest herd that we have been testing regularly, which consists of 60 cows. From each quarter, 10-cc. samples were drawn and smeared as indicated above.

The following standards were applied to the samples, which were examined without any knowledge of their origin:

The milk must be free (i) from all visible forms of micro-organisms, whether they be considered harmless or not; (ii) from leucocytes and fixed tissue cells, except those that may be cast off in the normal process of milk secretion; (iii) from polymorphonuclear leucocytes or other cells that occur during infection; (iv) from pathogenic organisms on cultural examination.

## RESULTS FOR COWS MEETING STANDARDS FOR NORMALITY

As a result of the examination of milk from the 60 cows, it was found that nine were giving normal milk. The differential cell counts are given in Table II. The figures represent the total number of each type of cell found in samples from the four quarters of each cow. Large and small forms of mononuclear cells are counted together. Many of the smaller ones showed little cytoplasm, and there were few of the large cells. The fat-bearing and epithelial lining cells are considered identical (Figs. 23, 24, 27). Epithelial scales come from the teat canal (Figs. 20, 21). Blue bodies are small, round, nuclear remains derived from the mononuclear cells (Figs. 19, 22). They stain a dark blue. Similar bodies, staining a paler blue, are believed to be globules of mucin. They are sometimes quite large, and are especially numerous in colostrum. Broken-down nuclear remains lacking cell walls were not counted.

TABLE II  
DIFFERENTIAL CELL COUNTS IN MILK FROM NORMAL COWS

Cow No.	Age, years	Number of cells						No. of fields counted
		Mono-nuclear	Fat-bearing and lining	Red	Epithelial scales	Blue bodies	Poly-nuclear	
1	3	42	28	0	0	Many	0	218
2	3	46	32	0	1	Numerous	0	211
3	3	26	25	1	2	Fairly numerous	0	226
4	2½	27	14	0	0	A few	1	241
5	2½	27	15	3	1	A few	0	222
6	3	19	15	2	Large strip	A few	0	197
7	5	5	7	15	1	None	0	260
8	3½	18	8	44	3	A few	0	226
9	3	21	15	5	4	A few	1?	238

A re-examination of cows No. 1, 2, 3, and 4 was made nine months later, and all four animals were found to be clean, as formerly, although No. 3 showed four polymorphonuclear leucocytes on a patch of mucinous material. One cow that showed a few polynuclears on the first test became clean on the second; another was clean on the first test, but showed diphtheroids and 17 polynuclears on the second. It is significant that when the data on the nine clean cows were compiled in Table II, it was found that all these animals were young.

Having defined normality in one herd, we attempted to find other normal animals by examination of two small herds, and three more cows have been added to the list.

## DISCUSSION

Although the personal equation must be considered in a histological method, the principal requirement in our standard of normal cow's milk is the absence of polymorphonuclear leucocytes, and these cells are unmistakable in their active form. This standard is difficult to attain. Therefore, freedom from

polymorphonuclears in milk is a very good sign. In support of our view, we quote from Maximow and Bloom (8), who state that in the lactating mammary gland in man "granular leucocytes are rare, and the presence of a noticeable number of them is always an indication of abnormal inflammatory changes, which have induced the migration of these elements from the blood vessels".

There are two rather puzzling conditions in cow's milk which should be mentioned here because of their bearing on normality. The first is the common occurrence of red blood corpuscles, accompanied by a certain number of leucocytes that are connected with the normal process of repair. The cause of haemorrhage into the udder is discussed later. The second condition is more difficult to explain. The mucinous state of the milk seen in newly calved cows may call forth large numbers of polymorphonuclear leucocytes, which will be found gorged with the mucin; later both mucin and leucocytes disappear. We have seen this condition described as normal. In our experience there is a variation in the amount of mucinous material produced, as only one or two quarters of the udder may be involved. This condition may disappear entirely. The same may be said of non-pathogenic organisms.

It would appear that normality in milk, as we have defined it, may exist at one period and not at another, which is what must be expected. Finally, it should be stated that in our attempt to define normality we have chosen a standard that would classify a very large percentage of cows as abnormal, judging by our results. We have not used such a drastic standard in grouping clean and diseased cattle in all our herds.

#### RESULTS FOR COWS NOT MEETING STANDARDS FOR NORMALITY

The standard for normality was not met by 51 cows of the herd of 60. Nine gave milk free from diphtheroids and other pathogenic organisms on culture, except for one occurrence of *S. aureus*; but leucocytes were numerous in five of these and polymorphonuclear cells were found in the others.

##### *Diphtheroid Carriers*

The remaining 42 cows, or 70% of the herd, showed epithelial scales infected with *Corynebacterium bovis*. Of these, 28 cows were infected with diphtheroids only, and 14 with diphtheroids associated with other organisms.

Twenty-eight cows were infected with diphtheroids not associated with any other organisms that could be detected by microscope or by culture. Summarized in age groups, these showed the following results:— the 9 oldest cows (6 to 13 years), few polymorphonuclear cells in 7, fairly numerous in 2; eight or more diphtheroid-bearing cells in 4, average of three in 5 (compare Table III): 6 cows (5 years), fairly numerous polymorphonuclears in 4, a few in 2; average of three diphtheroid-bearing scales in 5, more than eight in 1: 4 cows (3 to 4 years) few polymorphonuclears in 3, fairly numerous in 1; three diphtheroid-bearing scales in 3, several in 1: remaining 9 as below.

For comparison with the nine normal cows of Table II, in which cultures of milk samples were negative for pathogenic organisms, the findings for nine

young diphtheroid carriers are given in Table III. It can be seen that polymorphonuclear cells are more numerous in the latter group, although a few cows showed such small numbers that they would almost have appeared to be clean but for the infected epithelial scales. In Nos. 17 and 33 it was difficult to form a correct estimate, owing to the numbers of degenerate leucocytes present.

The polymorphonuclear counts are classified according to location of the cells in the smears, to show the larger numbers on the edge of the film. The epithelial scales were located with a low-power lens and then examined for diphtheroids with the oil immersion. It is probable that by following this method very few are overlooked.

TABLE III

POLYMORPHONUCLEAR CELLS IN MILK FROM COWS INFECTED WITH DIPHTHEROIDS

Cow No.	Age, years	Polymorphonuclear cells		Epithelial scales with diphtheroids
		Within smear	Edge of smear	
13	4	9	14	8
14	4	2	3	4
15	4	4	18	2
16	4	1	7	4
17	4	21	75	Several
25	3	1	18	1
26	4	4	8	6
31	2½	2	3	Several
33	2½	23 pyknotic	53 around scales	Numerous

*Approximately 200 fields counted for each cow.*

It appears that the older cows react less to diphtheroids than the younger. In this connection, attention might be drawn to cows Nos. 1, 4, and 5 (Table II), which became infected with diphtheroids since 1937, and in 1938 showed numbers of polymorphonuclear leucocytes. This occurred in the absence of infective organisms other than diphtheroids.

Fourteen cows were infected with diphtheroids associated with other organisms. Three of these had streptococcic mastitis. Seven showed large numbers of leucocytes associated with cocci. One showed cocci with few leucocytes. One had many leucocytes with contaminating organisms. Two had many leucocytes without organisms being found on culture.

In our herd of 60 cows 70% were infected, a much higher percentage than the 24% infected in 300 cows examined in the ordinary way. Most workers incline to the view that diphtheroids are harmless organisms. Martinaglia (7) stated that he had not found any evidence of leucocytic clumping around the bacilli. Hopkirk (6) suggests that micrococci and diphtheroids are not as toxic as some organisms and puts them in the same category.

We have encountered clumps of leucocytes surrounding groups of diphtheroids, and also polymorphonuclear cells ingesting the bacilli. Our evidence

points to an increase of polymorphonuclear leucocytes caused by the presence of diphtheroids (Figs. 16, 17, 21). But as regards pathological lesions, we have not found anything that points to an injurious effect on the udder. The diphtheroid-bearing scales are desquamated from the teat canal. The bacilli stain a deep purple with Hastings' and lie mainly on the surface of the scales. The scales themselves have an orange shade.

### Bacteriological Examination of Diphtheroid Strains

Twenty-five strains of diphtheroids were submitted to bacteriological examination. These were isolated from the milk of cows in a number of herds. They were seeded in litmus milk and in beef extract broth containing Andrade's indicator and lactose, maltose, dextrose, dextrin, glycerol, mannitol, saccharose, salicin, arabinose, raffinose, and inulin. No fermentation had occurred in any of these tubes after 11 days of incubation at 37° C. Litmus milk was slightly darker in colour after two weeks of incubation, and a marked grey sediment appeared.

Two recently isolated strains were seeded in sterilized milk. Intraperitoneal injection of 2 cc. of 4-day milk culture had no effect on two guinea pigs. The growth from a 48-hr. blood-agar slant injected intraperitoneally into two other guinea pigs was also innocuous. Subcutaneous injection of the same amount caused a slight dermal thickening after a couple of days and then disappeared.

These strains coincide with the description of *Corynebacterium bovis* sp. nov. (1).

### Staphylococcic Mastitis

The characteristic appearance of staphylococcic infections in milk under the microscope is of a multitude of polymorphonuclear leucocytes, many of which are ring-shaped (Fig. 1). They are evidently filled with a colourless material, which has pushed the nucleus over to the periphery of the cell. We have been unable to determine what this material is. The large number of these gorged-looking cells offers a very different picture to that of streptococcic mastitis. Staphylococci are not difficult to detect, as a rule; they may be found within clumps of leucocytes (Fig. 1). When phagocytosis is active they do not appear in large numbers within the cells (Fig. 3), while in some cases the leucocytes are able to control them completely and the cultures are sterile. Sometimes the polymorphonuclears will become so filled with cocci that they reach the bursting point (Fig. 15). These overloaded cells tend to gravitate to the edge of the film. We have not seen the macrophages ingesting either the cocci or the polymorphonuclear leucocytes in milk. Cocci are found in the lining cells from the ducts, and when this condition is observed it shows conclusively that the cocci are damaging the udder (Fig. 4). The cocci are very numerous in some samples, forming large clumps that spread out on all sides and almost obliterate the leucocytes. When the milk becomes "stringy", which is mainly due to shredding of the polymor-

phonuclear leucocytes, the cocci tend to disappear and the smear becomes almost free from cells except for a few of the mononuclear elements, which lie in the spaces between the broken-down cells. A few days later the fibrinous material disappears and there is a return to a more natural secretion from the udder. Unfortunately, the cocci are likely to reappear, accompanied by leucocytes.

During the course of the disease there are times when the leucocytes are few and the cocci numerous, lying free between the cells or crowding around them without being phagocytized. This would indicate a negative phase or lack of resistance on the part of the host. Staphylococci have been noted on the surface of epithelial scales from the teat canal. In colostrum the polymorphonuclears are active in picking up mucinous material. It is believed that they perform a useful function in getting rid of mucin, and also in phagocytizing cocci that may be present in the new milk. In any event, the leucocytes, mucin, and cocci disappear shortly. Mucinous material is not as commonly seen in staphylococcic infections as in streptococcic, while macrophage cells generally are equally numerous.

In view of the previous discussions on leucocyte counts, Table IV is given to show the changes in the leucocyte count in one animal (Case 2 of Table V). In the milk samples taken on June 25th, leucocytes were not numerous in the right quarters. In the left quarters, the leucocytes and cocci were five times more numerous. On August 26th, all quarters showed very large numbers of leucocytes and in three no cocci were seen microscopically. In the fourth quarter a few cocci were noted, and only a few colonies were found on culture.

TABLE IV

VARIATION IN NUMBERS OF ORGANISMS FOUND IN A CASE OF STAPHYLOCOCCIC MASTITIS  
(Case 2)

Date	Quarter of udder	Polymorphonuclears, %	Mononuclears		Lining cells, %	Fields counted	Total no. leucocytes counted	Staphylococci
			Small, %	Large, %				
June 25	RF	70	11	2	17	100	180	Free
	RH	66	13	2	19	100	217	Free and in leucocytes
	LF	57	23	4.5	15.5	20	160	In leucocytes
	LH	55.5	21	2.5	21	20	180	Free and in leucocytes
Aug. 26	RF	39.5	36.5	6.5	17.5	2	400	None
	RH	41.5	32	6	20.5	2	600	None
	LF	26	48	12.5	13.5	2	440	None
	LH	70.5	10.5	3	16	2	460	A few

Typical protocols recording the occurrence of leucocytes and cocci in three cases of *Staphylococcus aureus* infection are given in Table V, which shows how the disease may assume a chronic state. Case 1 became infected with *Streptococcus mastitidis* at the end of the period of observation reported here.

In Case 1 the staphylococci were missed once by microscopic examination when culture indicated they were present. In Case 2 the cocci were missed twice, and in Case 3, once. In the latter, both microscope and culture failed to show the organisms on Aug. 26.

TABLE V

EXAMINATION OF MILK FROM COWS WITH STAPHYLOCOCCIC MASTITIS

Case No.	Date	Leucocytes	No. affected quarters	Staphylococci found by	
				Microscope	Culture
1	Sept. 17, 1935	Numerous	3	In polys.	+
	Dec. 19	A few	4	In polys.	+
	Mar. 24, 1936	Many	4	Free and in polys.	+
	May 7	Many	3	Free and in polys.	+
	26	Many	3	Free and in polys.	+
	June 5	Many	4	Free and in polys.	+
	11	Many	4	None	+
	18	Many	1	In polys.	+
	25	Many	2	In polys.	+
	July 2	Many	3	Free	+
	9	A few	0	Free	+
	Aug. 26	Many	2	In polys.	+
	Jan. 11, 1937	Many	2	Free and in polys.	+
	April 13	Many	3	Free and in polys.	+
	July 13	Fairly numerous	2	In 1 quarter, also strep.	+
	Oct. 18	Many	1	Free	Strep. +
2	Dec. 19, 1935	None	0	—	—
	Mar. 26, 1936	A few	4	In polys.	+
	May 7	Many, fibrinous material	2	—	+
	12	Many	1	In polys.	+
	June 5	Many	1	—	+
	11	Many	2	Free	+
	18	Many	1	In polys.	+
	25	A few	4	In polys.	+
	July 2	Many	1	In polys.	+
	9	Many	2	In polys.	+
	Aug. 26	Many	4	A few in polys.	A few
3	Mar. 24, 1936	Fairly numerous	1	In epithelium	+
	May 7	Many	2	In polys.	+
	12	A few	1	Free and in polys., many	+
	June 5	Fairly numerous	1	—	+
	11	Fairly numerous	2	In polys.	+
	18	Many	3	In polys.	+
	25	Many	1	In polys.	+
	July 2	Many	3	In polys.	+
	9	Many	2	In polys.	+
	Aug. 26	A few	0	—	—
	Oct. 15	A few	0	In polys.	+

### Streptococcic Mastitis

A typical case of streptococcic mastitis, in which there are long chains of streptococci, is easily recognized microscopically (Fig. 8). These cases, however, are not very common. Others occur in which a few chains of cocci

are visible, or in which they are almost impossible to find. The appearance of a typical smear is different from that in other forms of mastitis, because the mononuclear cells (Fig. 2) are generally more numerous and among them are large macrophage cells filled with fat globules (Fig. 18). The total number of leucocytes is less than it is in staphylococcic infection. Mucinous material is commonly seen, even in cases of long standing. It is often ingested by the polymorphonuclears and stains a bright blue colour (Fig. 29). Lining cells are numerous, indicating injury to the udder. Clumping of the cells is usual; these form loose, irregular clumps, the polymorphonuclears being in separate groups with small and large mononuclears and macrophages around them. Blood corpuscles may be present also.

The mononuclears and macrophages show a different form of degeneration to the polymorphonuclears. The nucleus becomes fragmented, leaving round bodies of various sizes in the cell (Fig. 19). This has been seen so often that it seems worthy of note. In the acute stages there occurs an almost complete destruction of the polymorphonuclears, which shred to form fibrinous strands. In this process many small round bodies of chromatin are liberated and scattered over the smear.

The above microscopic picture suggests streptococcic infection even before the chains of cocci have been found. When these are rare, they may be seen in the polymorphonuclear cells. A difficulty arises here, for when cocci are visible in a cell one must make sure that there is definite chain formation (Fig. 13). Fortunately, there is often a prolongation of the chain outside the cell. The chains may also be seen on the surface of epithelial scales (Figs. 9, 20). It would appear that these scales afford neutral ground upon which organisms may develop, as we have repeatedly found streptococci and staphylococci growing upon them; especially is this true of diphtheroids, which occur there almost exclusively. Streptococci may also be found on desquamated lining cells.

Another indication that the case is streptococcic is the absence of other forms of organisms. In doubtful cases we have found Bryan's (4) method of incubating the sediment useful. It is not a bad plan to examine the four smears on each slide quickly at first, with a high-power dry lens. If streptococci are

TABLE VI

VARIATION IN NUMBERS OF ORGANISMS FOUND IN A CASE OF STREPTOCOCCIC MASTITIS

Quarter of udder	Polymorphonuclears, %	Mononuclears		Lining cells, %	Fields counted	Total no. leucocytes counted	Staphylococci
		Small, %	Large, %				
1	35	29	13	23	5	200	Free and in polys.
2	36	40	7	15	70	100	None
3	58	21	10	11	78	100	Numerous, free
4	64.5	18.5	10	7	10	330	Rare, in polys.

detected in one of the quarters, the slide can then be more carefully examined. Even the presence of abnormal numbers of leucocytes places a cow in the doubtful category, and failure to find streptococci in such a case merely means that the test should be repeated.

Table VI is appended to show the wide variation in the numbers of streptococci and to show the different reactions taking place simultaneously in the udder of an animal. The leucocytes were numerous in two quarters, 1 and 4; they were held in a mucinous network in quarter 4, and mucin was seen in the polymorphonuclears (Fig. 29).

Typical protocols recording the occurrence of leucocytes and cocci in three cases of *Streptococcus mastitidis* infection are given in Table VII.

TABLE VII  
EXAMINATION OF MILK FROM COWS WITH STREPTOCOCCIC MASTITIS

Case No.	Date	Leucocytes	No. affected quarters	Streptococci found by	
				Microscope	Culture
1	Dec. 19, 1935	—	—	—	—
	27	—	—	—	—
	Mar. 24, 1936	Many	2	In polys.	+
	May 7	Many	3	In polys.*	+
	July 9	A few	—	None	—
	Aug. 26	A few	—	Free	—
	Jan. 11, 1937	A few	—	In polys.*	—
	April 13	Many	1	None	+
2	July 13	A few	—	In polys.	+
	Dec. 19, 1935	—	—	None	—
	Mar. 24, 1936	Many	3	In polys.*	+
	May 7	Many	2	In polys.*	+
	26	Many	1	In polys.*	+
	July 9	Many	1	In polys.*	+
	Aug. 26	Many	3	2 chains	+
	Jan. 11, 1937	A few	—	None	+
3	April 15	Many	2	In polys.*	+
	July 13	Many	2	Free and in polys.	+
	Oct. 11, 1935	—	—	—	—
	Dec. 19	—	—	—	—
	Mar. 24, 1936	—	—	—	—
	May 7	Many	1	Staph.	+
	12	Many	1	Free	+
	July 9	Many, fibrinous	2	Short chains	+
	Oct. 15	A few	—	—	—
	Jan. 11, 1937	Many	1	—	+
	April 13	A few	—	—	+
	July 13	Many	2	Short chains	+

\* Doubtful whether streptococci or staphylococci.

† Non-pathogenic forms of cocci.

### *B. coli* Mastitis

Milk samples from 10 cases of *B. coli* mastitis have been examined. In four cases the most outstanding abnormality was the large number of lining cells from the udder (Fig. 7). In one of these, it looked as though a considerable

part of the lining had been sloughed out; in the other three, the lining cells were numerous. Red blood corpuscles were also noted. The polymorphonuclear leucocytes were degenerating and shredding in six cases. Bacilli were common in eight, chromatin granules were numerous in three cases (Fig. 6). Mucin was noticeable in two cases, bacilli being very numerous in the mucinous patches.

### ***Corynebacterium pyogenes* Mastitis**

Seven lots of samples from cows infected with *Corynebacterium pyogenes* have been examined. The most prominent feature under the microscope was the large number of organisms seen; among them occasional coccoid forms occurred. The polymorphonuclear phagocyte many of the organisms, then break down and liberate them all over the field. These broken-down polymorphonuclears gather in large tangled skeins, and chromatin granules are noticeable. Lining cells and crenated red blood cells are numerous.

### **Phagocytosis and Destruction of Cocci**

In a discussion of resistance to diseases of the udder, it is well to consider it briefly as indicated by the effect of the invading organisms on the cells of the udder, and by their effect on the milk. The organisms that grow and multiply in the udder damage the cells of the alveoli and ducts, as seen by the presence in the milk of degenerate lining cells associated with these organisms. There is reason for believing that the damage is partly, perhaps mainly, superficial. The leucocytes that are attracted to the affected part migrate into the lumen of the ducts and attack the organisms. The latter may be living on the surface of the tissues or in the descending stream of milk, where they are in an ideal medium for growth. The attempt of the leucocytes to attack these organisms, with subsequent loss to the animal of both, must be responsible for a very heavy drain of leucocytes from the blood. Yet effective resistance to infection in the udder is indicated clinically, because one frequently sees only one or two quarters of the udder affected at one time. With these general observations in mind, the microscopic observations will be discussed.

In support of the view that the polymorphonuclear cells produce antibacterial substances, we have repeatedly noticed in contaminated milk samples that the contaminants grew feebly or not at all in the quarters in which many leucocytes occurred. The milk from leucocyte-free quarters of the same animal would be heavily contaminated.

When large numbers of leucocytes are present in milk, it often means that few or no micro-organisms will be found microscopically or culturally. This has been observed in both staphylococcal and streptococcal infections. It is followed by resolution, when the leucocytes disintegrate and disappear. On other occasions numerous leucocytes and organisms have been seen degenerating simultaneously. The process of resolution, as observed in milk, differs from that described for the serous cavities, where the macrophages

phagocyte the damaged polymorphonuclear leucocytes. In milk we have not observed the macrophages phagocytizing the polymorphonuclear cells, but they do pick up the small mononuclears (Fig. 28), often in large numbers. They also ingest mucin, red corpuscles, and nuclear remains.

In staphylococcic mastitis, infected leucocytes are not as numerous as one would expect, except in those cases in which there is low resistance and the leucocytes are few in number. Commonly, small clumps of five to six polymorphonuclears will be encountered with a few cocci scattered outside them. At other times large, gorged cells will be seen quite replete with cocci.

In streptococcic mastitis the evidences of resistance are easier to detect than they are in staphylococcic, because when chain formation becomes irregular it is readily seen. In several samples the cocci appeared to be granular wherever the chains touched or lay across a polymorphonuclear cell (Fig. 11), and normal where they did not touch. From these observations it appeared that the lytic agent was inside the cells rather than in the surrounding fluids. In other instances the streptococci were among clumps of polymorphonuclears showing signs of degeneration (Fig. 14), the chains were short, and the cocci granular and irregular in size, suggesting an extracellular effect upon them (Figs. 10, 12). Atypical forms have been noticed repeatedly. This would suggest that they are meeting opposition in their growth and may have lost a certain amount of virulence. We have seen little evidence of encapsulation in our samples.

In conclusion, certain general statements may be made regarding the microscopic examination of milk sediment. When resistance is high there will be many leucocytes and few organisms present. When many organisms and few leucocytes are found, it is probable that a new invasion of the udder will take place. Active phagocytosis indicates a return to normality.

### **Leucocytic Reactions Occurring in Non-Infected Swellings of the Udder**

Injuries to the udder occasionally occur that are followed by resolution without infection, as indicated in the following brief record. Cultures of all milk samples were negative.

#### *Record of Heifer*

When this heifer calved there was a hard swelling in the LH quarter. Blood clots were milked out. Cow first examined about three weeks later. The LH quarter was fairly hard but not painful.

*Day 1.* RF, RH, and LF quarters clean. LH quarter: Many monos, polys, macros, clumping. Red blood corpuscles numerous.

*Day 7.* RF, RH, and LF quarters clean. LH quarter: Numerous monos and lining cells in clumps. Not many polys. Macrophages picking up red cells, which had become less numerous. No organisms seen.

*Day 14.* RF, RH, and LF quarters: A few polys, monos, and lining cells. LH quarter: Fairly numerous monos, lining cells, and macros. Polys

more numerous on islands of mucin which they were ingesting. No organisms seen. Swelling reducing.

*Day 22.* RF, RH, and LF quarters clean. LH quarter: Fairly numerous monos, lining cells, and small aggregations of polys. No organisms seen. Observations ceased.

We have recently encountered another case that was similar to the above. This serves to show that in cows such cases do occur, though they are probably not common.

### Red Corpuscles in Milk

Red blood cells are of common occurrence in milk, and they are often unaccompanied by any signs of infection. The enormous distension of the udder that often occurs probably causes the blood vessels to rupture; in samples of colostrum we have seen much blood, which supports this explanation. The red corpuscles usually appear well shaped and normal. In severe infections, like *B. coli* or *C. pyogenes*, the appearance of the red cells is different; they are crenated and often stain badly (Fig. 15). In a few samples the milk has had a dark red coloration with few corpuscles visible under the microscope, which is caused by rupture of the cells and liberation of haemoglobin. Macrophage cells picking up the corpuscles have been repeatedly observed. It would appear that when milk is bloody it is frequently caused by mechanical injury to the udder, and in a smaller number of cases it is connected with disease.

### Chromatin Staining Granules and Shredding of Leucocytes

Chromatin granules may occur in large numbers, sometimes covering the entire slide (Fig. 6); fibrinous strings are generally associated with them. They represent a breaking-down of the polymorphonuclear leucocytes and also of the lining cells from the milk ducts. Both these degenerative products are found when there is complete destruction of the cells. In the early stages of the process, formation of the chromatin granules is quite noticeable before the cells break down (Fig. 5). These changes have been noted frequently in our records.

Muñ (10), writing about the destruction of cocci in man, says "the polymorphonuclear leucocytes are seen to be in a degenerated condition, their nuclei being fragmented and changed into deeply staining chromatin globules". It is evident, therefore, that the changes in milk closely resemble those that occur in the tissues in other parts of the body.

### Calcium Phosphate Deposits in the Teats and Udder Tissue

According to several authorities on pathology, calcium is deposited in the tissues as a result of some form of injury. In the udder, bacterial infections are common and mechanical injuries also occur frequently. The parts involved are soft, secreting tissues, which are not intended to be handled roughly. Possibly a combination of infection and mechanical injury may cause the deposits in the walls of the teats, but in the udder it is more likely that infection

is responsible, because large numbers of calculi have been found quite high in the outer portions of the walls (Fig. 25). According to Muir (10), dead cells and old collections of pus may become the starting point of calcification. Fatty tissues also undergo calcification in some cases. In an indurated teat a complete ring of calcium was found just below the milk cistern. Dr. H. M. LeGard informs us that in his practice he has frequently been called on to remove small calculi from the teat canal. Sweet, Miller and Graves (12) record that many lobules showed numerous small concretions or milk calculi in the alveoli, *post-mortem* in the udders of five cows. They did not comment on the origin or significance of these calculi in the udder.

The recent Australian Milk Commission Report (11) does not list any reference to calcium deposits in milk nor to the calculi in the tissues.

In autopsies on dairy cattle at the Toronto abattoirs a number of calculi were detected, and in one case about a gram of small calculi was picked out of the tissues. These calculi were tested by H. W. Lemon of this Foundation, who reported they were probably a mixture of di-calcium and mono-calcium phosphate.

#### DETECTION OF CALCULI IN MILK

It was found much simpler to detect calculi in the tissues than in the milk sediment. Even now, though a considerable number of cases have been recorded, it is often difficult to determine the nature of certain bodies encountered on the slides. Microscopically, the calculi show concentric rings and appear dark in colour with crenated edges, closely resembling those found in the udder tissues (Fig. 26).

When calculi are plentiful in a milk smear they may alter the aspect of the stained preparation, giving it a more open, granular appearance, and when the finger is passed over the surface it gives the sensation of sand paper. Calculi have been repeatedly observed in certain of our cows, sometimes accompanying disease, at other times in healthy animals. Further examinations will have to be made before anything more definite can be said about the frequency of their occurrence in milk.

#### Colostrum

Colostrum being the first milk secreted by glands that have previously been non-functional, it was decided to examine the udder in pregnant heifers. Sections were cut, giving information as to the type of cells that would be found in the new milk. In a young heifer, pregnant about three to four months, it was found that the two hind teats were patent and milk was being secreted in these quarters. The front teats were still closed. The alveoli showed secreting and non-secreting portions. In the secreting tissues the alveoli were circular and the walls thicker, the lining cells and nuclei rounder,

than in the non-secreting areas. These findings would support the statement of Porcher and Panisset, quoted by Bourgeois (2), that colostrum is composed of the remains of milk previously secreted.

In colostrum there is much debris and large numbers of nuclear remains, which we have called blue bodies in our records (Fig. 22). These are sometimes accompanied by a multitude of mucin-like globules. The figures of colostrum bodies given by Maximow and Bloom (8) bear a resemblance to Fig. 23 in this paper, which represents the nuclei of lining cells from the ducts accompanied by strands of fat globules. These nuclei are commonly encountered. A pale-blue-staining nucleolus can usually be detected within the nucleus, but the cytoplasm that surrounds the cells in the alveoli has disappeared in the colostrum. A few small mononuclear cells and numbers of macrophages with fat droplets occur in some specimens. Polymorphonuclear cells have been noted, actively phagocytizing the mucinous globules, in the absence of micro-organisms. Red blood corpuscles are numerous. They are irregularly distributed, being very plentiful in one quarter and scarce in another. They mean little as far as the identification of colostrum is concerned.

We have examined some 20 samples of colostrum. The smears are generally coloured a dark blue with Hastings' stain, owing to the mucinous material present. When leucocytes are numerous the colour changes to red. Apparent infections that rapidly cleared up have been found in several cases, and we have subsequently passed some of these cows as clean. It is generally stated that colostrum only persists a few days, though in a few of our samples we have been able to detect nuclear remains and mucinous material for more than a fortnight.

### Acknowledgments

We wish to express our thanks to Dr. H. B. Speakman, Director of the Foundation, for giving us time and freedom to do this work, and for helpful suggestions. Dr. H. M. LeGard has willingly co-operated with us in his own time, performing the clinical examinations and giving us interesting specimens. We are also indebted to several members of the staff of the Foundation for friendly assistance, especially Mr. H. W. Lemon for calcium determinations.

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## PLATE I

FIG. 1. *Staphylococcal infection. Note ring-shaped polymorphonuclears.* FIG. 2. *Leucocytes in streptococcal mastitis. The mononuclear cells predominate. In such cases the milk may be microscopically and culturally sterile.* FIG. 3. *Polymorphonuclear leucocyte containing two pairs of cocci.* FIG. 4. *Lining cells from the milk ducts infected with staphylococci.* FIG. 5. *Degenerating leucocyte containing two rows of chromatin globules.* FIG. 6. *Chromatin globules scattered over field. These are derived from the breakdown of the polymorphonuclear cells.* FIG. 7. *B. coli mastitis. Note numbers of lining cells.*

## PLATE II

FIG. 8. *Actively growing chains of streptococci from milk sediment.* FIG. 9. *Streptococci growing on an epithelial scale, with a ring of polymorphonuclears surrounding them.* FIG. 10. *Atypical chains of cocci growing free in milk. No leucocytes are attacking them. The club-shaped ends and enlarged grains suggest that the medium is not favourable.* FIG. 11. *The chain of cocci that crosses the central cell shows degenerative changes where it touches the cell. Beyond the cell, the cocci appear normal. Degenerate cocci are also visible in the upper cell.* FIG. 12. *The streptococci are being destroyed in the lower cell, and above them a few small chains appear normal.* FIG. 13. *Single polymorphonuclear cell with short chains of streptococci. In samples where cocci are scarce, the finding of one or two infected cells is a great help in diagnosis.* FIG. 14. *Streptococci being actively phagocyted by the polymorphonuclears.* FIG. 15. *The large central polymorphonuclear cell is gorged with micrococci. Red blood corpuscles are numerous and the leucocytes are degenerating.*

## PLATE III

FIG. 16. *Epithelial scales from the teat canal bearing diphtheroids, some of which are free, others being phagocyted around the edges.* FIG. 17. *Clump of polymorphonuclears phagocytosing diphtheroids.* FIG. 18. *Macrophage cells that have ingested fat droplets. These fat-bearing cells are common in streptococcal mastitis, but occur in lesser numbers in other forms of mastitis.* FIG. 19. *Nucleus of leucocyte breaking into round masses that occur in large numbers in colostrum.* FIG. 20. *Epithelial scale bearing diphtheroids and chains of streptococci.* FIG. 21. *Characteristic appearance of diphtheroid-bearing scale.* FIG. 22. *Colostrum. Nuclear remains (called blue bodies in the text).*

## PLATE IV

FIG. 23. *Nuclei of lining cells from udder with fat droplets. They have lost their cytoplasm. These cells are numerous in various affections of the udder and in colostrum.* FIG. 24. *Lining cell surrounded by a ring of fat globules which are attracted to the cell.* FIG. 25. *Calcium concretions in section from udder. Note crenated borders.* FIG. 26. *Calcium deposit in milk sediment. Note similarity in shape to Fig. 25.* FIG. 27. *Normal lining cells that have broken away intact from the walls of the udder.* FIG. 28. *Macrophage cell filled with small mononuclear cells.* FIG. 29. *Polymorphonuclear cells ingesting mucinous material.*

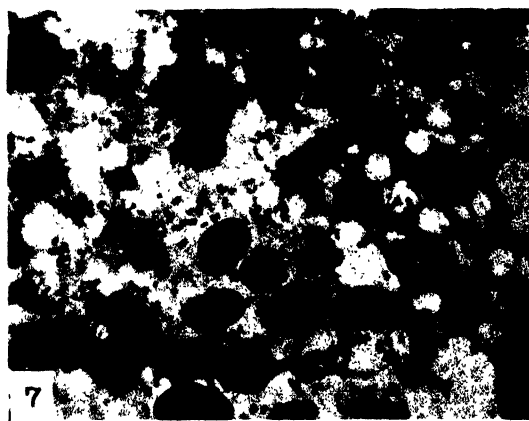
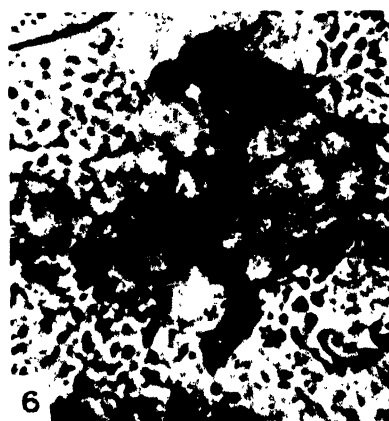
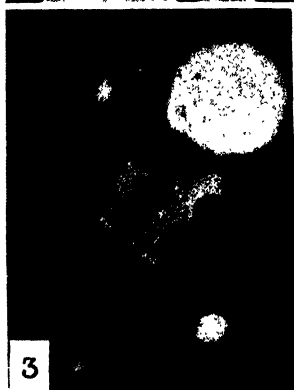
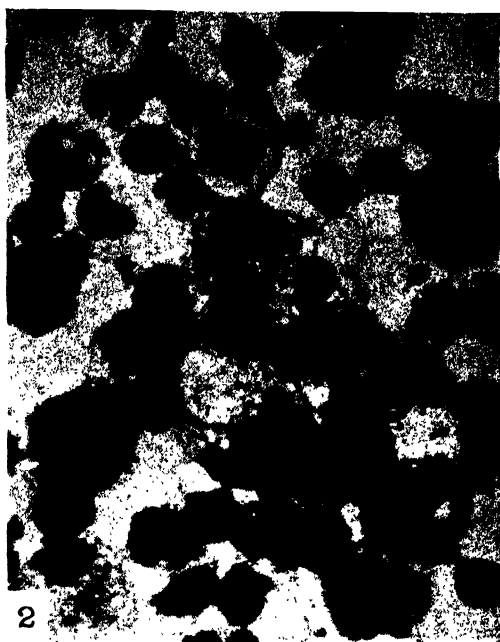
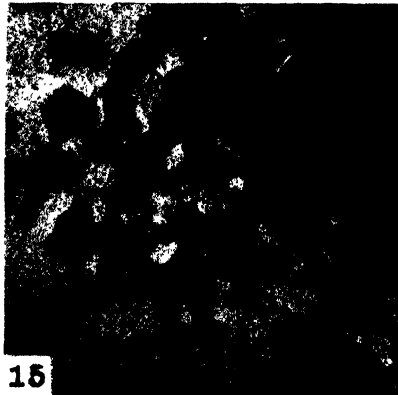
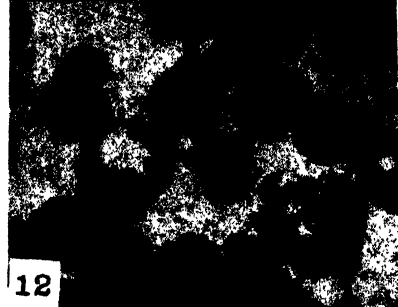
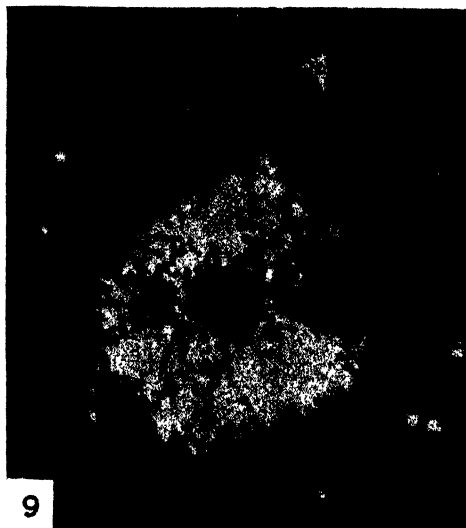


PLATE II



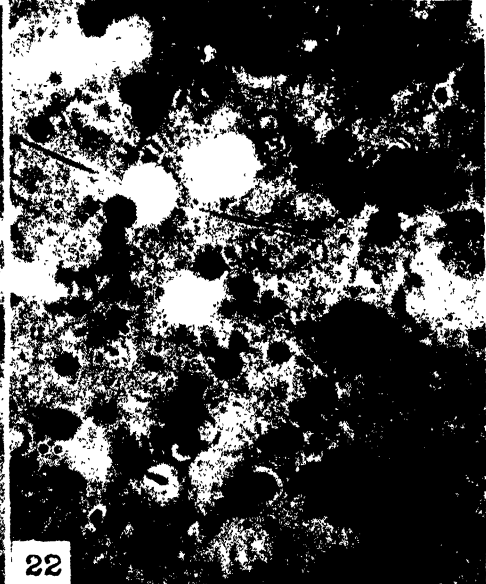
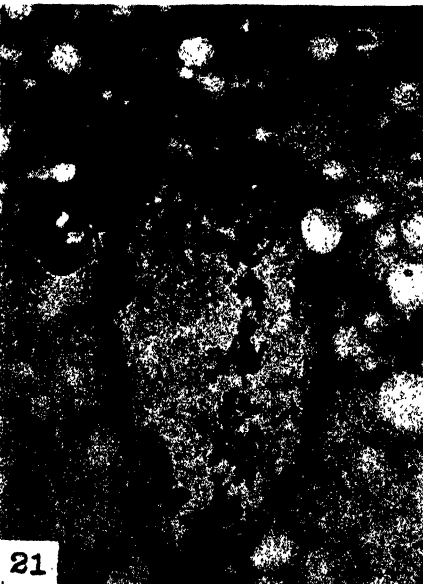
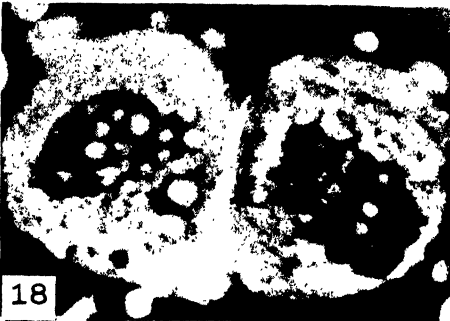
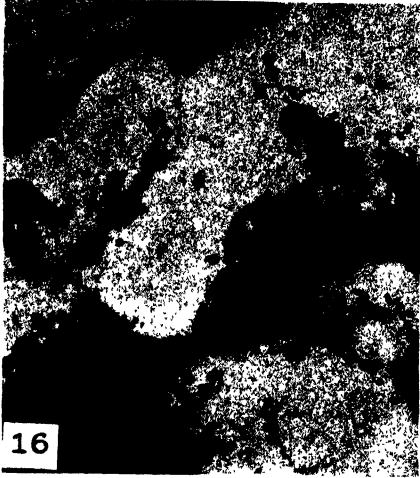
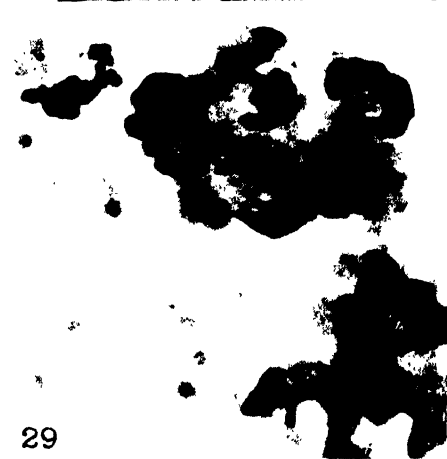
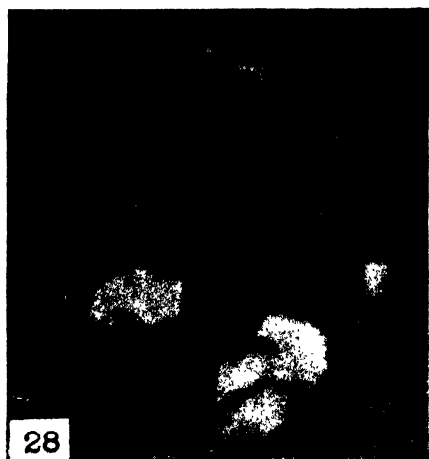
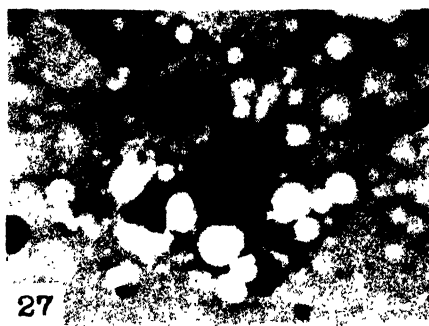
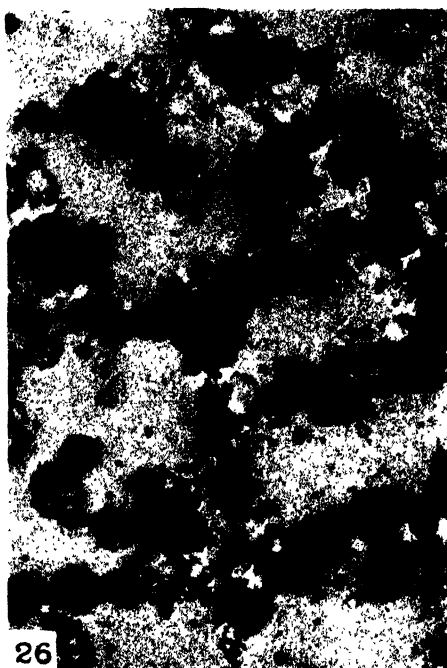
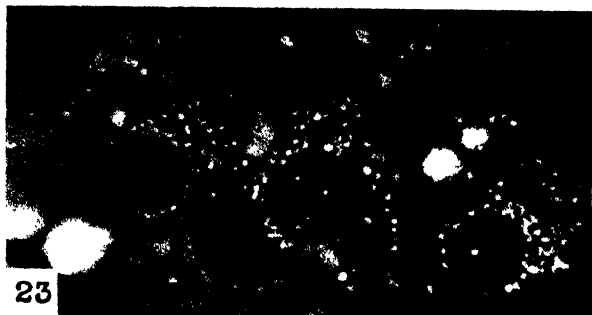


PLATE IV



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## STUDIES ON THE ENDOPARASITIC FAUNA OF TRINIDAD MAMMALS

### VI. PARASITES OF EDENTATES<sup>1</sup>

BY THOMAS W. M. CAMERON<sup>2</sup>

#### Abstract

Of the internal parasites from two species of anteaters and an armadillo from Trinidad, B.W.I., five species of nematodes are described as new to science, viz., *Lauroia trinidadensis*, *Delicata pseudoappendiculata*, *Longistriata cristata*, *L. urichi*, and *Pintonema tamandua*.

There are three edentates found in the island of Trinidad, B.W.I. These are two anteaters and an armadillo.

*Tamandua longicaudata* (syn. : *T. tetradactyle*) or "Tamandua" is also called the Lesser Anteater to distinguish it from the Ant-bear. This latter animal is absent from the island, however, and the Tamandua is the largest species found there. It is essentially arboreal but is often found on the ground.

Seven individuals were available for examination.

*Cyclopes pygmaeus* (syn. : *C. didactylus*) or Silky Anteater, is known locally as the Sloth; the true sloth, however, is absent from Trinidad, although found on the adjacent mainland. The Silky Anteater is a tiny, arboreal creature, seldom descending to the ground on which it walks with difficulty.

Only two specimens were available for study and a single female tricho-strongyle was the sole helminth recovered.

*Tatusia novemcincta* or Armadillo is the third edentate found in Trinidad; elsewhere this species is called the nine-banded armadillo. Its distribution extends into the southern United States. It is quite strictly terrestrial and is the only edentate found on other West Indian islands, occurring on Tobago and Grenada; it has possibly been introduced by man into Grenada at least.

Three armadillos were available from Trinidad.

I have again to express my indebtedness to the late Professor Urich and to Mr. Fitzgerald. They collected and shipped to Canada the entrails of the animals from which these specimens were obtained. The material was

<sup>1</sup> Manuscript received June 28, 1939.

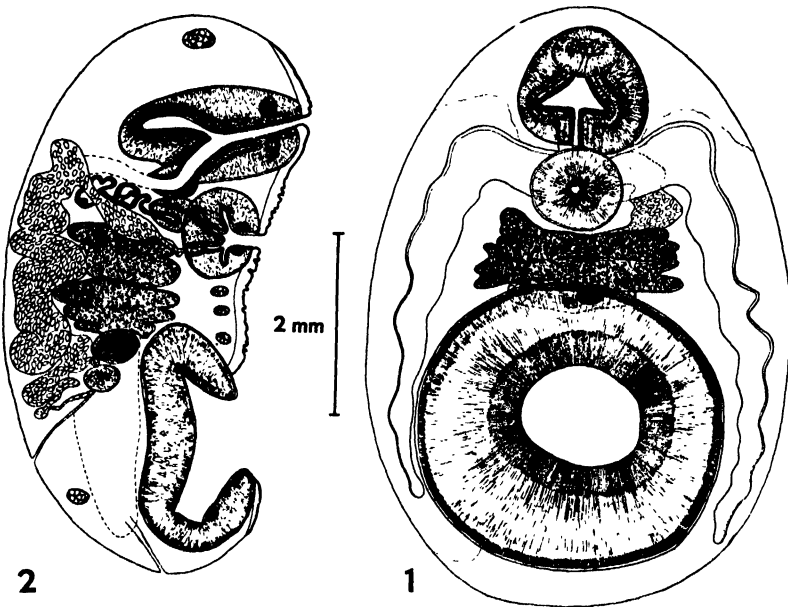
Contribution from the Institute of Parasitology, McGill University, Macdonald College, Quebec, with financial assistance from the National Research Council of Canada.

<sup>2</sup> Professor of Parasitology, McGill University, and Director, Institute of Parasitology, Macdonald College, Canada.

preserved in formalin in bulk, and then wrapped in formolized cloth, sealed in tins, and sent by parcel post. It arrived in excellent condition. After being soaked in water, the entrails were opened, and the parasites collected and preserved in a standard mixture of formolized glycerine alcohol.

*Stichorchis giganteus* (Diesing, 1835) Travassos, 1922

A number of amphistomes was found in the intestine of one Tamandua. Some of these were stained and mounted as whole mounts, others were serially sectioned. Figs. 1 and 2 are reconstructions of these from the ventral and lateral view-points. The specimens measure about 6 mm. long by 4.5 mm. wide by 2.75 mm. thick. They are oval in outline and flat on the ventral face. The mouth opening is at the anterior end of the ventral surface, the acetabulum at the posterior and the genital opening just in front of the middle. The cuticle on the ventral surface is much thicker than that over the remainder of the body.



FIGS. 1 and 2. *Stichorchis giganteus*. FIG. 1. Reconstruction from serial sections of entire animal from ventral aspect. FIG. 2. Reconstruction from lateral aspect of medial serial sections.

The acetabulum is massive, with a diameter of about 2.5 mm. The mouth is surrounded by an oral sucker and pharynx fused together; there is a pair of large posterior diverticula to the pharynx, one on each side. The oesophagus is short and has a muscular sphincter, quite distinct from that of the pharynx. The caeca are massive and extend to the posterior region of the body.

The genital opening is surrounded by a massive genital sucker into which open the male and the female ducts. The testes are slightly dendritic, tandem and close to each other. They are much wider and broader than long, and they occupy most of the space in the middle of the body between the anterior

margin of the acetabulum and the genital sucker. The vas deferens is much coiled in front of the anterior testis and forms a seminal vesicle. This is situated just behind the genital sucker but slightly to one side. Before it enters the sucker, it forms an S-shaped loop, and this part is surrounded by a glandular structure.

The ovary is small and spherical and lies just posterior to the posterior testis. The portion next to the oviduct is much more closely granular than the remainder. A shell gland lies just posterior and to one side of the ovary. The oviduct is narrow and much convoluted and appears to be filled with spermatozoa. Laurer's canal runs from this point to the dorsal surface of the body, opening directly behind the acetabulum. The uterus is thrown into a number of lateral loops on the dorsal aspect of the body. The uterus joins the genital sucker posterior to the vas deferens. There are numerous yolk follicles scattered about the body, mostly posterior and ventral to the other genitalia, but some in the anterior region of the body.

Two species of *Stichorchis* are known—one from the beaver, which is quite different, and one from porcine animals in South America, the peccary being apparently the original host. The recent re-description by Vas of *S. giganteus* from the pig in Brazil, shows that the present specimens are very closely related to it. The differences that exist are mainly those of dimensions and proportions. In general the sizes recorded for porcine material are larger; the present specimens were fixed in formalin *in situ* and may have shrunk considerably, while host environment may equally account for smaller size. Moreover, the Collared Peccary occurs in Trinidad, although *Stichorchis* has not yet been recorded from it there. Accordingly, these specimens from the *Tamandua* are referred to *S. giganteus*.

*Oöchoristica* spp. inq.

Fragments of tapeworms belonging to this genus were found in one *Tamandua* and one armadillo. It was impossible to identify the species in either case.

*Gigantorhynchus echinodiscus* (Diesing, 1851)

This thorny-headed worm was present in each *Tamandua* examined, sometimes in considerable numbers. It does not differ in any material manner from Travassos' description (1917).

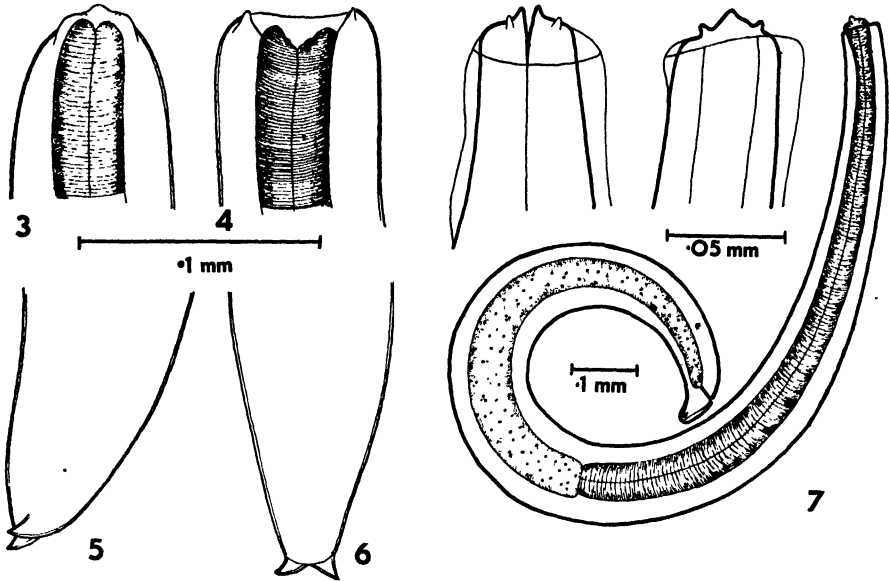
*Strongyloides* sp.

A single specimen of *Strongyloides* was found in the small intestine of a *Tamandua*. It was 3.6 mm. long. The oesophagus was 1.1 mm. long, and the vulva was situated 1.1 mm. from the tail, which was 0.05 mm. long and bluntly pointed. In view of the fact that a single formalin-fixed specimen is available, no attempt is made to assign it to a species.

*Acanthocheilonema* sp.

A single filaria worm was found on the outside of the intestine of one *Tamandua*. It was a female, 42 mm. long and 0.25 mm. wide. No transverse

striations were observed. The mouth (Figs. 3 and 4) is in a hollow with a pair of dorso-ventral lips. Each of these carries three papillae, the central one of which is on a projection; each lip accordingly is trilobed. The oesophagus is 1.3 mm. long and the anus opens 0.15 mm. from the tip of the tail. The tail is rounded with two lateral triangular projections (Figs. 5 and 6), one on each side; the apex of each triangle is directed ventrally, outwards and backwards.



FIGS. 3-6. *Acanthocheilonema* sp. FIG. 3. Head of female from lateral aspect. FIG. 4. Head of female from ventral aspect. FIG. 5. Tail of female from lateral aspect. FIG. 6. Tail of female from ventral aspect.

FIG. 7. *Physaloptera* sp. Entire larval *Physaloptera* with insets showing details of head from ventral (upper right) and lateral aspects.

The nerve ring is 0.2 mm. from the mouth opening with the excretory pore just in front of it. The vulva is situated 0.55 mm. from the mouth. The long slender ovejector joins the uterus just behind the end of the oesophagus. The ovarian tubules are double.

This parasite closely resembles the description of *Acanthocheilonema perstans*, a filariid parasite of man (which also occurs in Trinidad). It is, however, shorter (42 mm. as against 70 to 80 mm. long) and wider (0.25 mm. as against 0.12 mm. wide). The monkey fauna of Trinidad consists of a Red Howler (*Alouatta insularis*) and a Capucin (*Cebus appella*). I have had no opportunity of examining the viscera of either species, but no parasites have been reported from them. However, members of the genus *Acanthocheilonema* occur in South American monkeys elsewhere. The present specimen is considerably smaller than these also.

The absence of any males prevents a specific diagnosis although it certainly belongs to this genus.

*Physaloptera* sp.

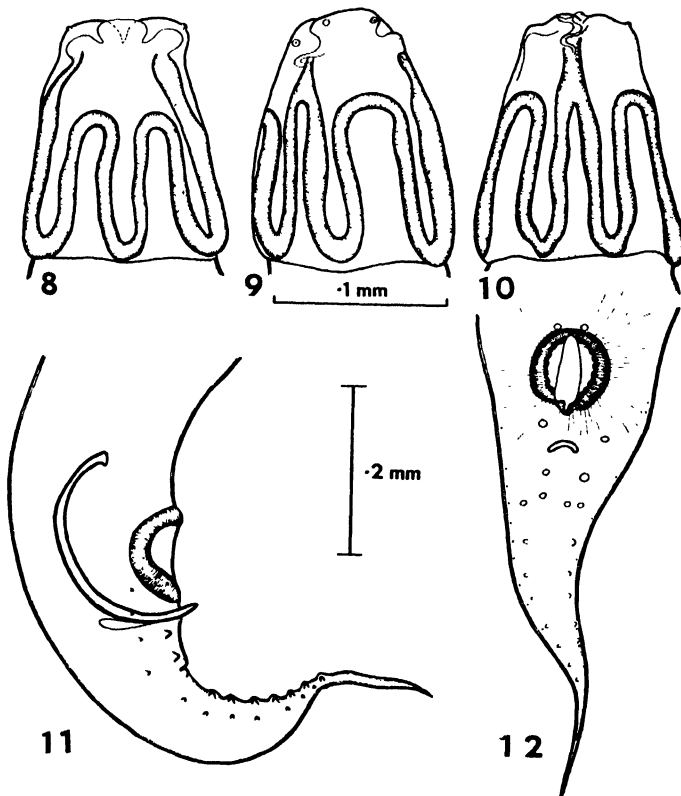
Five specimens of a larval *Physaloptera* were found in two *Tamandua* and one in an armadillo (Fig. 7). These all appear to be the same species. They are all small (2 mm.) with the oesophagus more than half the body length. The mouth has two projecting lips, each carrying three papillae. The tail is short and conical. There is no trace of any genitalia.

It is impossible to refer this species to any existing form. It is almost certainly abnormal in these edentates.

*Aspidodera binansata* Railliet and Henry, 1913

A considerable number of specimens, always in association with the next mentioned species, was found in the large intestine of all three armadillos; in one animal they occurred in the small intestine as well.

Both sexes are of about the same size, the average length being 5.5 mm. and the maximum width 0.5 mm. The body is finely striated throughout and lateral crests run from near the mouth to the anus. The cervical papillae lie in depressions in these crests.



FIGS. 8-12. *Aspidodera binansata*. FIGS. 8-10. Head from dorsal, lateral, and ventral aspects; in Fig. 9 the dorsal face is next to Fig. 8. FIGS. 11 and 12. Tail of male from lateral and from ventral aspects.

The head is similar in both sexes (Figs. 8, 9, and 10). The mouth is surrounded by three lips, each of which carries two small papillae. The dorsal lip is trilobed, the two others are simple. The head carries three sets of festoons, the function of which is not obvious although they may be glandular in nature. The dorsal festoon has two re-entrant loops, the others, one each. The outer limbs of each festoon unite to form an anterior projection; from each projection a small S-shaped duct runs forward to the inter-labial space. The contents of the festoons are granular and they move under pressure.

The head is distinctly separated from the remainder of the body by a constriction.

The oesophagus, which is 1.3 mm. long, has a distinct posterior dilation. The excretory pore, the nerve ring and the cervical papillae all lie about the middle of its length. The female tail is long and slender with a pair of small caudal papillae 0.35 mm. from the tip. The tail is 0.8 mm. long.

The entire genitalia is very compact and the ovaries are much coiled. The uteri are divergent but unite to form a long muscular ovejector which is directed forward to the vulva. The vulva lies just in front of the middle of the body. Each uterus contains about 40 eggs, which measure about  $60\mu$  by  $40\mu$  and have a thick shell.

The male tail is somewhat more sharply pointed than is that of the female. A conspicuous sucker is present just in front of the ano-genital opening (Figs. 11 and 12). This sucker is not complete posteriorly. Two papillae lie just in front of it and four rows of papillae run from its posterior margin towards the tip of the body. There is considerable irregularity both in the number and disposition of these papillae.

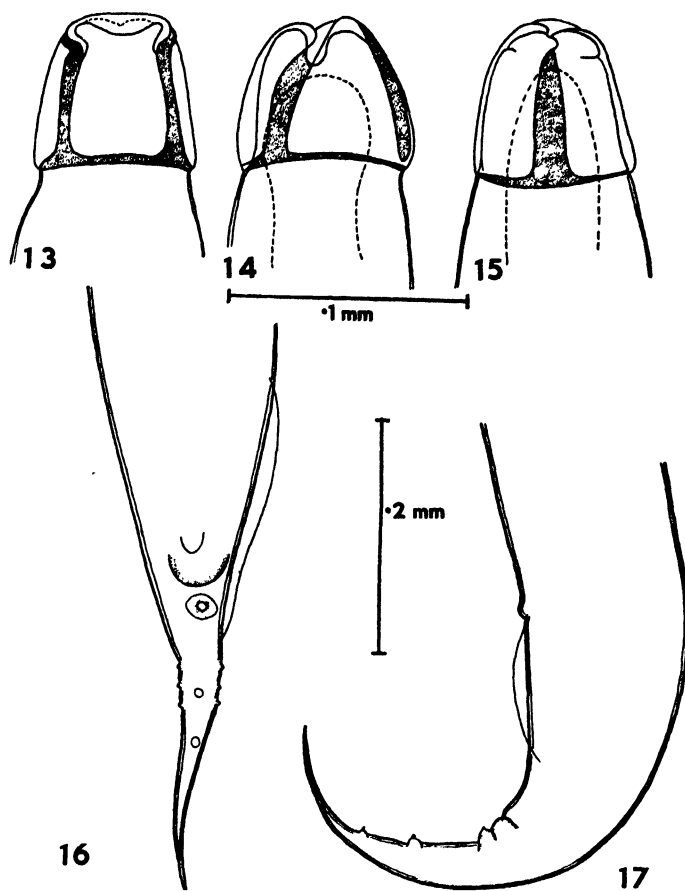
Two plain tubular spicules and an accessory piece are present. The spicules are 0.32 mm. long and the accessory piece about 0.1 mm. long.

*A. fasciata* (Schneider, 1866) has, *vide* Proença, a single re-entrant festoon on the dorsal face whereas this species has two. This would refer it to *A. binansata* Railliet & Henry, 1913, which Proença records from the same host in Brazil.

*Lauroia trinidadensis* sp. nov.

This parasite in considerable numbers was found in association with the previous species. The female is slightly larger (6 to 7 mm.) but the male is about the same size (5.5 mm.). The constricted head region in this species is very much smaller but there is a wide mouth opening, the base of which possesses a number of minute teeth. The head is surrounded by three flat cuticular plates (Figs. 13, 14, and 15). The dorsal one is rectangular with antero-lateral projections. The others are triangular, with interlocking antero-ventral margins. These plates are unstriated and no papillae were observed.

A narrow striated crest runs on each side of the body from just behind the head to the rectum.



FIGS. 13-17. *Lauroia trinidadensis* sp. nov. FIGS. 13-15. Head from dorsal, lateral, and ventral aspects; in Fig. 14, the dorsal face is towards Fig. 13. FIGS. 16 and 17. Tail of male from ventral and from lateral aspects.

The oesophagus is about 0.7 mm. long. The portion of the oesophagus within the head cap is separated from the remainder, which has a distinct bulb with a valvular apparatus. There are no intestinal diverticula at the oesophageal-intestinal junction. The excretory pore and nerve ring lie just about the middle of the oesophagus but no cervical papillae were seen.

The female tail is 0.75 mm. long and sharply pointed. It is conspicuously striated.

The vulva is at the junction of the anterior and middle thirds. It communicates with a long backwardly directed ovejector which splits into divergent parts. Each of these is continued as divergent uteri and ovarian tubules; each uterus with its tubules is confined to its own portion of the body. The eggs measure  $65\mu$  by  $45\mu$  and have thick shells.

The male tail is more sharply and abruptly pointed than is the female tail (Figs. 16 and 17). There is no sucker present but there is an asym-

metrical swelling or membrane on the left side of mature forms. This is not seen in young males. There are two central small, and one large, adanal papillae. Another papilla lies just anterior to the ano-genital opening. The tip of the tail is striated and carries three small lateral papillae and two medial post-anal papillae.

There is no gubernaculum. The spicules are subequal, the left measuring 0.8 mm. and the right 0.9 mm. The spicules end in fine points but are composed of a series of minute cuticular rings contained within a sheath.

Proença has recently (1938) described *Lauroia travassosi* from this host and *Dasytus sexcinctus* from Brazil. His specimens are not only larger than the Trinidad forms but differ from them in other details. The head plaques of the Trinidad species do not have the posterior appendices of the type; the spicules are sub-equal and much longer, and there is no true caudal sucker present. For these reasons this species is regarded as different and the name *L. trinidadensis* sp. nov. is proposed for it.

*Trichocephalus* sp. inq.

A single whipworm—a female in poor condition—was recovered from one Tamandua. Owing to this, no attempt has been made to describe it or refer it to a species.

*Graphidiops costalimai* Lent & Freitas, 1938

This species is common in the Tamandua. The female is 6 mm. long and the male about 4 mm. long. The body is finely striated. The head is slightly swollen and the striations are more conspicuous.

The mouth is surrounded by six minute papillae but is otherwise simple (Fig. 18).

The oesophagus is 0.5 mm. long, with the excretory pore about its mid-point and the nerve ring slightly behind this.

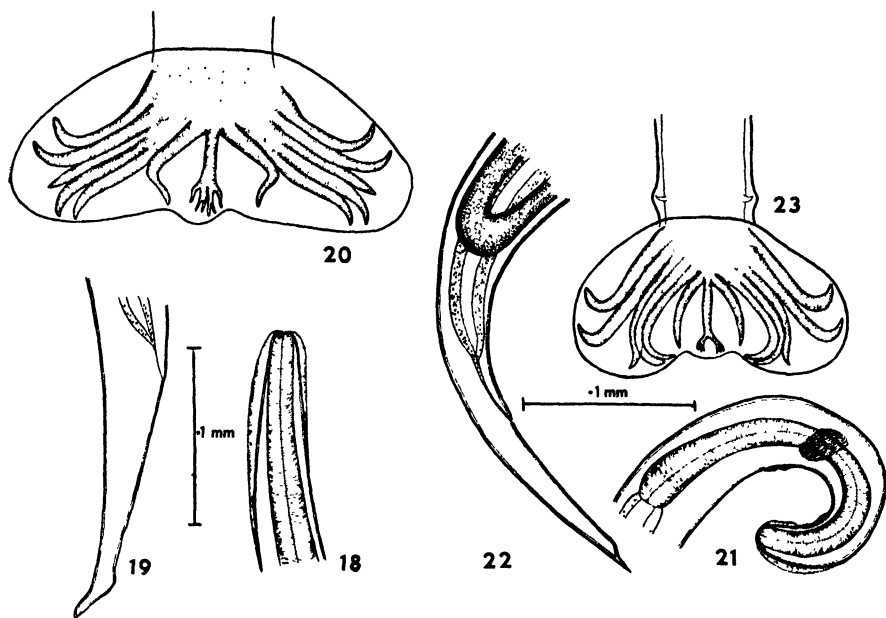
The female genitalia is double and the ovejectors are opposed, although the vulva is situated near the posterior end of the body, 1 mm. from the tail. There are 10 to 14 eggs in each uterus.

The tail is elongated and the anus is 0.15 mm. from the tip (Fig. 19).

The male bursa (Fig. 20) is small with a crenated border and a small dorsal lobe. The dorsal ray bifurcates towards its tip, each branch re-dividing and the medial sub-branches being again divided. The externo-dorsal ray runs parallel with the lateral rays for half its length, when it suddenly turns towards the dorsal. All the other rays are sub-equal and parallel for most of their lengths, diverging only slightly at the tips; the externo-lateral alone does not reach the margin of the bursa.

The spicules are equal, about 0.4 mm. long and bifurcate at the ends, one bifurcation being blunt and the other sharp.

This species agrees well with Lent and Freitas' account of *Graphidiops costalimai* from the Brazilian Tamandua. It would seem that the female of their "*Graphidiops* sp. I." is actually the female of this species.



FIGS. 18–20. *Graphidiops costalimai*. FIG. 18. Head. FIG. 19. Tail of female. FIG. 20. Male bursa. FIGS. 21–23. *Delicata pseudoappendiculata* sp. nov. FIG. 21. Head. FIG. 22. Tail of female. FIG. 23. Male bursa.

*Delicata pseudoappendiculata* sp. nov.

This species is also very common in the Tamandua. It is quite small, the female measuring 3.4 mm. and the male, 2.8 mm.

The body is transversely striated and usually forms two loose spirals in its anterior portion. In addition, the head is always bent ventrally and the cuticle on its anterior portion is swollen, giving it a somewhat asymmetrical appearance (Fig. 21).

The oesophagus is 0.23 mm. long. The nerve ring is about its middle and the excretory pore, which is conspicuous, behind this.

The ovarian tubules in the female both originate in the anterior part of the body and are almost without convolutions. The ovejectors and uteri are opposed, however, and situated in the posterior part of the body, the vulva being 0.5 mm. from the tip of the tail. The anterior uterus has 4 to 5 eggs, the posterior one only 2 to 3. The ovejectors are of the *Trichostrongylus* type.

The tail is gently tapered, suddenly narrowing at its tip to a fine dorsal spine. The anus is 0.1 mm. from the tip (Fig. 22).

The bursa of the male (Fig. 23) is relatively small with a small dorsal lobe and an accessory membrane. Small pre-bursal papillae are present. The dorsal ray is slender and Y-shaped, each part being divided into three processes. The externo-dorsal and the lateral rays are slender. The lateral rays are parallel to each other but the tip of the externo-lateral is bent ventrally, whereas the other two bend dorsally. The ventral rays lie close together

with their tips bent ventrally. They are more massive than the other rays and the ventro-ventral appears to be split.

The genital cone has two small papillae. An accessory piece, about half the length of the spicules, is present. The spicules are short stout rods, 0.1 mm. long. They are split at their ends and the inner face of the medial arms is provided with teeth.

This species appears to belong to the genus *Delicata*, being very closely related to the species *D. appendiculata* described by Travassos in 1928 from the Tamandua from Brazil. It differs from this species in the smaller size of the female, and in the ventral flexure of the head. No vulva flap was seen, although the lips are prominent. The spicules appear to have only two points, but the characteristic teeth noted by Travassos in *D. appendiculata* are present.

It differs from *D. khalili*, the second species found in this host by Travassos in Brazil, by its general smaller size, the shape of the spicules, the partial separation of the ventral rays. It resembles it more closely, however, in the shape of the female tail.

The name *Delicata pseudoappendiculata* sp. nov. is accordingly proposed for it.

*Fontesia fontesi* Travassos, 1928.

This is a common species in the Tamandua. It is easily recognized by its slender size and its asymmetrical head with the ventral prolongation to the mouth (Fig. 24). There is a slight pseudo-swelling at the head end caused by a constriction of the body but not of the cuticle. The tail of the female is conspicuously striated and ends in a very fine elongated point (Fig. 25). Two swellings are found on the ventral side of the body at the base of this extension. The male bursa is comparatively large with long, slender rays (Fig. 26). The two spicules are short and ornate.

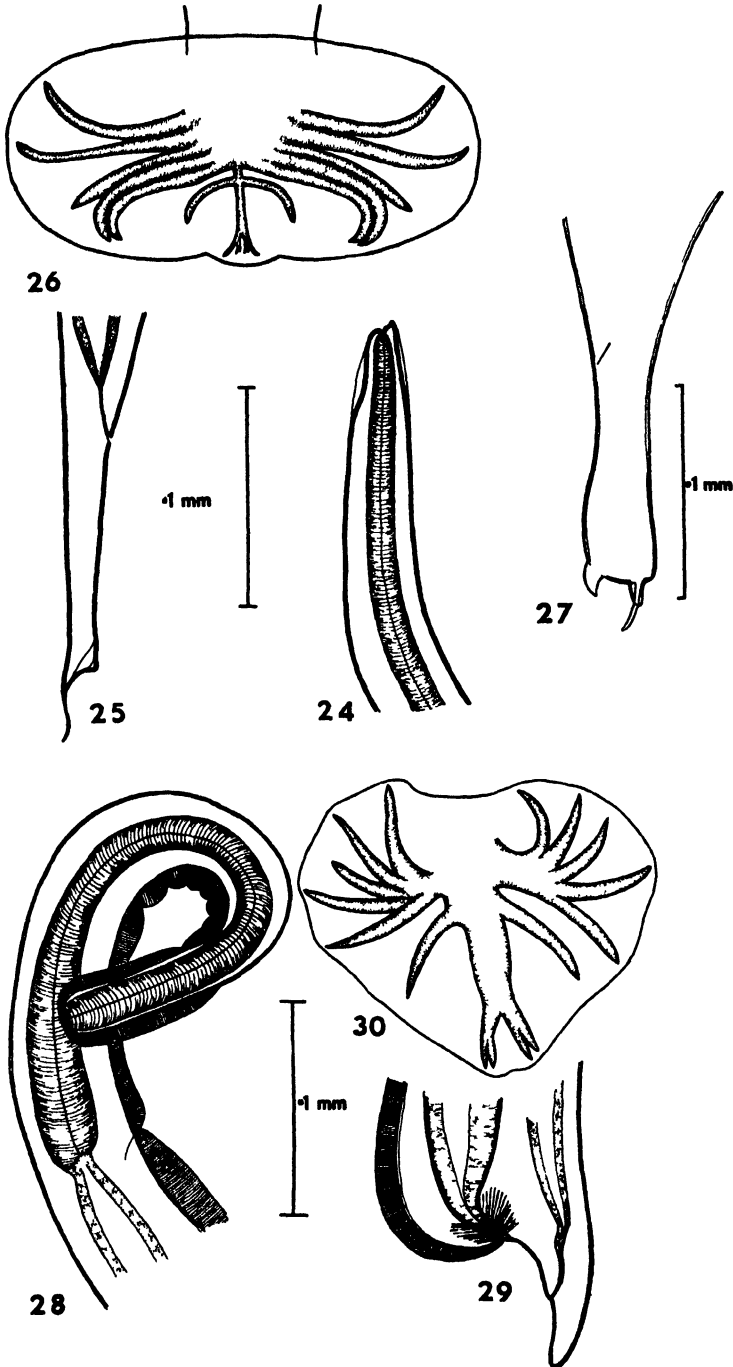
The Trinidad specimens do not differ in any significant points from those described by Travassos from the same host in Brazil.

*Bradypostrongylus inflatus* (Molin, 1861) Travassos, 1937

A single female specimen of this species was found in a Tamandua. It was 10 mm. long, with transverse striations throughout the entire length of the body; lateral alae occur in the medial region only. The oesophagus is 0.75 mm. long and there are large backwardly-directed cephalic papillae. The vulva, which is situated 2.0 mm. from the posterior end of the body, is a transverse slit, guarded by a backwardly directed flap. The uteri are divergent and the genital tubes, as in *Haemonchus*, spiral around the intestine.

The anus is 0.1 mm. from the blunt tail (Fig. 27). The tail itself carries three processes, a slender dorsal and two conical, more massive, projections; these arise from a flat base.

This species has been previously described by Travassos from this host from Brazil.



FIGS. 24-26. *Fontesia fontesi*. FIG. 24. Head. FIG. 25. Tail of female. FIG. 26. Male bursa. FIG. 27. *Bradypoststrongylus inflatus*. Tail of female. FIGS. 28-30. *Longistriata cristata* sp. nov. FIG. 28. Head. FIG. 29. Tail of female. FIG. 30. Male bursa.

*Longistriata cristata* sp. nov.

This species from the stomach of the Tamandua is about 8 mm. long in the case of the female and 6 mm. in the male. The maximum width is 0.07 mm. The cuticle is finely striated and there is a conspicuous broad ventral crest, which is also striated and runs from head to tail. The cuticle around the head end is inflated and striated. The mouth is simple (Fig. 28). The oesophagus, which is only slightly swollen posteriorly, is 0.33 mm. long.

The genital system in the female is single and the vulva is situated just in front of the anus at the posterior end of the body. This is abruptly truncated, the actual tail being short and blunt (Fig. 29). The eggs are  $65\mu$  by  $40\mu$  and there are 40 to 50 in the uterus, in a single row, lying obliquely across it.

The male bursa (Fig. 30) is complete and triangular with no dorsal lobe, but a small dorsal notch is present. The main dorsal stem is massive and bifurcated only at the end; each bifurcation ends in two large digitations. The externo-dorsal rays are relatively small and arise from near the base of this stem. The other rays are spread out fan-wise and are about equal in size.

The spicules are 0.5 mm. long and slender, but each ends in a finger-like process. There were neither accessory piece nor prebursal papillae present but just in front of the bursa is a small secondary crest, which is quite short, in addition to the main ventral crest which is continued to the head.

This species seems to belong to the genus *Longistriata*, and to the sub-genus *Longistriata*, but it differs from the described forms in the triangular bursa and the massive dorsal ray. It is accordingly regarded as a new species with the name *Longistriata cristata* sp. nov.

*Longistriata urichi* sp. nov.

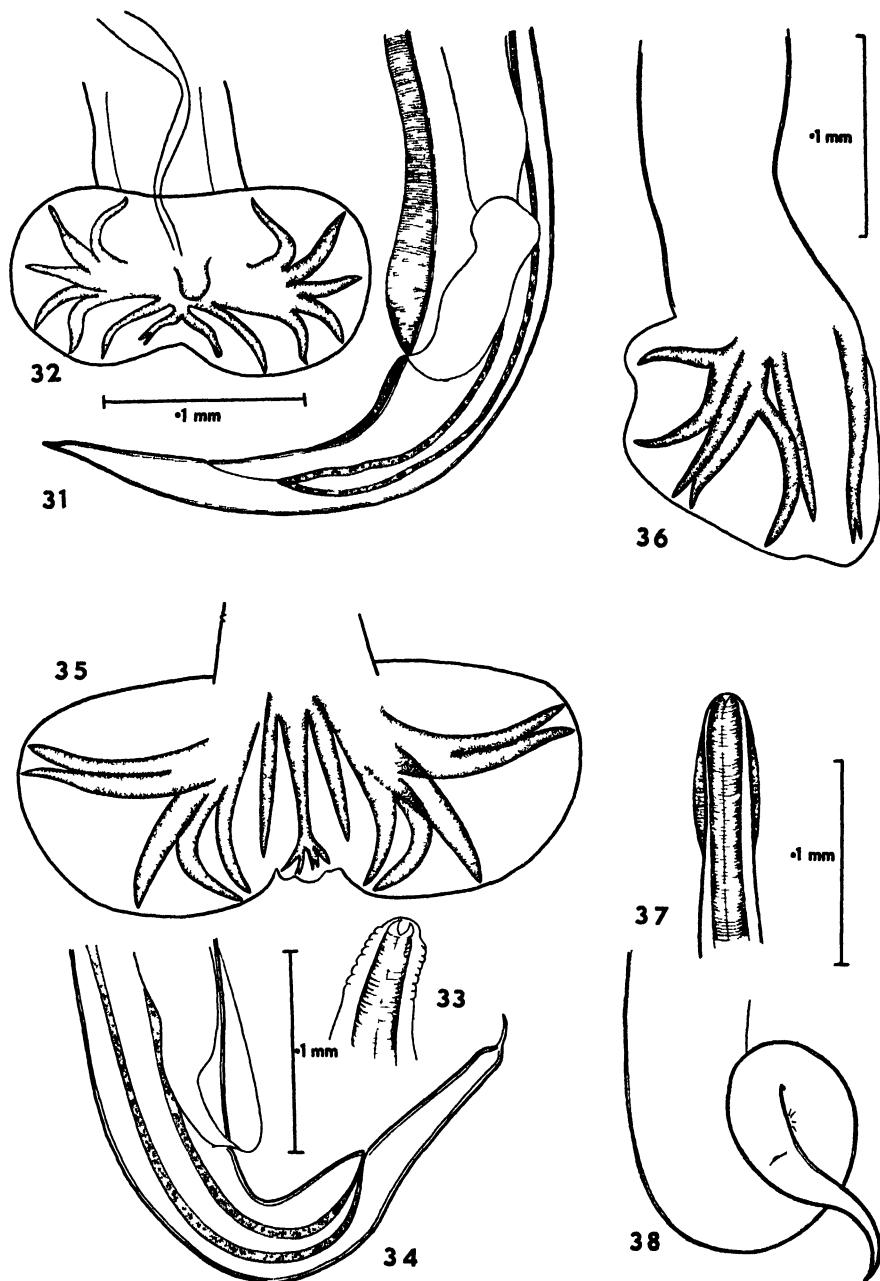
These nematodes from the Tamandua were coiled in a loose spiral and consequently their length was difficult to determine; one typical female, however, measured 2.5 mm. long with an oesophagus measuring 0.32 mm.

The head is simple and slightly swollen; the mouth is a simple pore. Lateral crests are present and towards the tail of the female, a ventral crest also.

The female genitalia is single and opens to the exterior about 0.22 mm. from the tip of the tail (Fig. 31). A massive ovejector is present. The eggs measure about  $60\mu$  by  $40\mu$ . The anus is 0.09 mm. from the tip of the sharp, acute tail.

The male bursa is small, without a dorsal lobe, the dorsal ray being split almost to its base (Fig. 32). All the rays are spread out so that their apices are about equidistant from each other. The dorsal rays end in two small digitations; all the others are slender and pointed. The ventro-ventral rays are curved inwards. There is no accessory piece and the spicules are slender, needle-like, poorly chitinated structures, measuring 0.15 mm. each.

This very large genus has recently been revised by Travassos (1937) and he has recognized several sub-genera. This species belongs to the sub-genus *Carolinensis*, characterized by the long spicules and the externo-dorsal ray rising independently of the dorsal. Only two species occur in this sub-genus,



FIGS. 31 and 32. *Longistriata urichi* sp. nov. FIG. 31. Tail and terminal genitalia of female. FIG. 32. Male bursa. FIGS. 33-35. *Pintonema tamandua* sp. nov. FIG. 33. Head. FIG. 34. Tail and terminal genitalia of female. FIG. 35. Male bursa. FIG. 36. *Pudica pudica*. Male bursa from side. FIGS. 37 and 38. *Heligmosominae* sp. inq. FIG. 37. Head. FIG. 38. Tail of female.

viz., *carolinensis* and *musculi*, both described by Dikmans (1935) from rodents in the United States. The first species (from deer mice or prairie meadow mice) is rather larger, possesses longer spicules and an accessory piece in the male and has a shorter blunter tail. The second species (from *Mus musculus*) is similarly larger, with longer spicules, the dorsal ray split only a third of its length in the male, and a shorter tail in the female. The present species is accordingly regarded as undescribed and the name *Longistriata urichi* sp. nov. is proposed for it.

*Pintonema tamandua* sp. nov.

A number of specimens of this species was found in Tamandua.

The female is 3.8 mm. long and the male only slightly smaller. Both are about 0.1 mm. thick. The body is of a uniform width and nearly cylindrical except at the ends. The anterior end tapers rapidly and is square cut. Behind the oesophagus, the body is coiled in one or two coils. The entire body is striated transversely but it has also a number of longitudinal ridges. The cuticle at the head end is swollen and more coarsely striated.

There is a prominent mouth cavity (Fig. 33) and a relatively short oesophagus (0.32 mm.) with a slight posterior enlargement.

The female genitalia is single. The uterus contains 8 to 10 ova,  $60\mu$  by  $40\mu$  in size. The ovejector is short but voluminous with a sudden narrow constriction joining it to the ovary. The vulva is situated 0.2 mm. from the tip of the tail; it is a transverse slit, anterior to which is a ventral cuticular expansion, which is without striations (Fig. 34).

The tail of the female is always bent ventrally at a point between the vulva and the anus. The anus is about 0.1 mm. from the tip of the tail. The tip of the tail narrows to form a small filariform appendix.

The male bursa (Fig. 35) is large with a small dorsal lobe. The margin is crenate. The dorsal ray is slender and bifurcates near its tip, each bifurcation dividing almost immediately into two further divisions; the median division is split again. The ventral and the lateral groups are each compact and separate from each other. The ventral rays are long, slender, and close together.

The genital cone has two prominent papillae. The spicules are long and slender, 0.29 mm. in length, with the tips slightly swollen and apparently bifurcated. A small gubernaculum is present.

This species appears to belong to the genus *Pintonema* created in 1935 by Travassos to include four species from *Dasytus novemcinctus*. The present species differs from all of these, however. It is larger than all except *P. pulchra*, which it most closely resembles; the female of this species has not yet been observed and so no comparison is possible. The prominent mouth cavity is not described for it, however, prebursal papillae are absent, the terminal digitations of the dorsal ray are S-shaped, and the externo-dorsal rays widely spread out; the spicules also are less slender and not swollen terminally. For these reasons, therefore, this species is regarded as new and

the name *Pintonema tamandua* sp. nov. is proposed for it. No species of *Pintonema* were found in the armadillo from Trinidad.

*Pudica pudica* (Travassos, 1921) Travassos & Darriba, 1929

A few examples of this species, hitherto recorded only from rodents, were found in a Tamandua.

The head end is sharply constricted from the rest of the body and is conspicuously striated transversely. The mouth is simple.

There are about ten longitudinal ridges on the body, each ridge carrying the fine transverse striations of the body.

The oesophagus is 1.5 mm. long.

The female is 2.5 mm. and the male 2.25 mm. long. The tail of the female is short and stumpy. The genital system is single and opens posteriorly just in advance of the rectum. The single uterus is long and contains a dozen or so eggs. These are thin-shelled and measure about  $60\mu$  by  $40\mu$ .

The male bursa is relatively voluminous (Fig. 36). The main dorsal stem is split for about two-thirds of its length to form two long dorsal rays; each ends in a bifurcation. The externo-dorsal ray is widely separated from the dorsal and lies close to the dorso-lateral. This, in turn, is widely separated from the other two lateral rays, which lie close together. The ventral rays are widely separated from each other.

There is no accessory piece. The spicules are 0.23 mm. long and each ends in a pair of simple points.

This parasite was originally described by Travassos (1921) from *Dasyprocta agouti* from Brazil under the name of *Viannaia pudica*. As this host also occurs in Trinidad, it is probable that the Tamandua is an abnormal host, a circumstance which would account for the small number collected. In spite of this fact, there are no significant differences to note from Travassos' original description.

HELIGMOSOMINAE sp. inq.

A single female heligmosome was found in the Pigmy Anteater (Figs. 37 and 38). It was a small specimen, 4 mm. long, with the body covered throughout with very fine striations. The head is simple, with a cuticular enlargement more coarsely striated than the remainder of the body. The mouth is simple and the oesophagus slender and 0.3 mm. long. The excretory pore, at the base of the oesophagus, is guarded by two small lips. The genital system is simple and the vulva is 0.1 mm. in front of the anus, which in turn is about the same distance from the tip. The tail is elongated and tapers to a fine point.

As no trichostongyles have been yet recorded from this host and as no males were available, it cannot be referred to any species. It belongs, however, to the subfamily Heligmosominae and as such it is placed on record.

TRICHOSTRONGYLIDAE sp. inq.

A single female was found in an armadillo but was in such a poor condition that further identification was impossible.

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Section D

INDEX TO VOLUME 17

Authors

- Baker, F. C.**—Land and freshwater mollusca from western Ontario, 87.
- Bowstead, J. E. and Sackville, J. P.**—Studies with a deficient ration for sheep. I. Effect of various supplements. II. Effect of a cobalt supplement, 15.
- Cameron, T. W. M.**—Investigations on Trichinosis in Canada. II. A further survey of the incidence of *Trichinella spiralis* in hogs in eastern Canada, 151.  
Studies on the endoparasitic fauna of Trinidad mammals. VI. Parasites of Edentates, 249.
- Fraser, C. M.**—Hydroids of the western Canadian Arctic region, 1935-1937, 59.
- Froman, D. K.**—See Swales, W. E.
- Garrard, E. H. and Lochhead, A. G.**—A study of bacteria contaminating sides for Wiltshire bacon with special consideration of their behaviour in concentrated salt solutions, 45.
- Griffiths, H. J.**—Observations on the bionomics of ova and miracidia of *Fasciola hepatica* Linn., in eastern Canada, 205.
- Gwatkin, R.**—See Hadwen, S.
- Hadwen, S. and Gwatkin, R.**—The detection of abnormal cow's milk by microscopic methods, 225.
- Hart, J. F. L.**—Cumacea and Decapoda of the western Canadian Arctic region, 1936-1937, 62.
- Hastings, R. J.**—The biology of the meadow nematode *Pratylenchus pratensis* (de Man) Filipjev 1936, 39.
- Honeyman, A. J. M.**—Lacustrine investigations in the Gaspé peninsula, 212.
- Komarov, S. A.**—See Langstroth, G. O.
- Komarov, S. A., Langstroth, G. O., and McRae, D. R.**—The secretion of crystalloids and protein material by the pancreas in response to secretin administration, 113.
- Langstroth, G. O.**—See Komarov, S. A.
- Langstroth, G. O., McRae, D. R., and Komarov, S. A.**—The synthesis and secretion of protein material by the pancreas, 137.
- Lochhead, A. G.**—See Garrard, E. H.
- Lyster, L. L.**—Parasites of freshwater fish. I. Internal trematodes of commercial fish in the central St. Lawrence watershed, 154.
- McRae, D. R.**—See Komarov, S. A., Langstroth, G. O.
- Munro, J. A.**—Food of ducks and coots of Swan Lake, British Columbia, 178.
- Parnell, I. W.**—Studies on the bionomics and control of the bursate nematodes of horses and sheep.  
VI. On the lethal effects of some nitrogenous chemicals on the free-living stages of *Sclerostomes*, 68.  
VII. The effect of some substances, used in the control of farm and household pests, on the free-living stages of *Sclerostomes*, 187.

**Sackville, J. P.**—See Bowstead, J. E.

**Selye, H.**—Some blood chemical changes during recovery from exhaustive muscular exercise, 109.

**Swales, W. E. and Froman, D. K.**—An apparatus for measuring the "flash" thermal death point of microscopic animal organisms and its use with ova of *Ascaris lumbricoides*, 169.

**Timonin, M. I.**—Pathogenicity of *Beauveria Bassiana* (Bals.) Vuill. on Colorado potato beetle larvae, 103.

**White, W. H.**—Determination of nitrite, nitrate, and chloride in cured meat and curing pickle, 125.

**Winkler, C. A.**—Colour of meat.

I. Apparatus for its measurement, and relation between pH and colour, 1.

II. Effect of desiccation on the colour of cured pork, 29.

Dew-point hygrometer for use at low temperatures, 35.

Tenderness of meat. I. A recording apparatus for its estimation, and relation between pH and tenderness, 8.

Section D

INDEX TO VOLUME 17

Subjects

**Acanthochellonema** sp., 251.

**Acetodextra amiura**, 163.

**Acipenser fulvescens** as host of trematodes, 167.

**Alfalfa**

- ash supplement, Effect on sheep of, 23.
- hay and oats ration, Effect on sheep of, 24.
- meal supplement, Effect on sheep of, 23.

**Ambloplites rupestris** as host of trematodes, 167.

**Ameiurus nebulosus** as host of trematodes, 167.

**American Coot**, Food of, 182.

**American Pintail**, Food of, 182.

**Ammonium carbonate**, Effect of, on the free-living stages of *Sclerostomes*, 77.

**Ammonium chloride**, Effect of, on the free-living stages of *Sclerostomes*, 77.

**Ammonium nitrate**, Effect of, on the free-living stages of *Sclerostomes*, 82.

**Ammonium sulphide**, Effect of, on the free-living stages of *Sclerostomes*, 84.

**Amnicola**, 91.

**Anas platyrhynchos**, See Mallard, Common.

**Anguilla rostrata** as host of trematodes, 167.

**Anguispira**, 100.

**Aniline**, Effect of, on the free-living stages of *Sclerostomes*, 70.

**Animal organisms**, An apparatus for measuring the "flash" thermal death point of microscopic, 169.

**Anodonta**, 88.

**Anodontoides**, 89.

**Anteater**

- Lesser, See *Tamandua longicaudata*.
- Silky, Parasites of the, 249.

**Aplexa**, 99.

**Arctic region**, Western Canadian  
Cumacea and Decapoda of the, 62.  
Hydroids of the, 59.

**Argis dentata**, 66.

**Armadillo**, Parasites of the, 249.

**Ascaris lumbricoides**, Single-celled ova of, "Flash" thermal death point of, 173.

**Aspidodera binansata**, 253.

**Azygia longa**, 165.

**Bacillus coli** in milk, 238.

**Bacon**, Wiltshire, Bacteria contaminating sides for, 45.

**Bacteria**

- contaminating sides for Wiltshire bacon  
Classification of types of, 49.  
Effect of concentrated salt solutions on, 45.  
Nitrate-reducing, in pickling brine, 54.

**Beauveria Bassiana**, Pathogenicity of, on Colorado potato beetle larvae, 103.

**Bicarbonate** in pancreatic juice, 116.

**Blood**

- chemical changes during recovery from exhaustive muscular exercise, 109.
- chloride values of rats after exhaustive muscular exercise, 110.
- corpuscle concentration in rats after exhaustive muscular exercise, 111.
- corpuscles, Red, in milk, 241.
- sugar values of rats after exhaustive muscular exercise, 110.

**Bonneviella gracilis** n. sp., 59.

**Brachydiastylis**

- nimia, 65.
- resima, 65.

**Bradypoststrongylus inflatus**, 258.

**Breed method** of counting leucocytes in milk, 228.

**Bucephalopsis pusilla**, 154.

**Buffle-head Duck**, Food of, 182.

**Bulimnea**, 93.

**Bunodera sacculata**, 161.

**Calcium**

- in pancreatic juice, 116.
- supplement, Effect on sheep of, 21.

- Calcium cyanide**, Effect of, on the free-living stages of *Sclerostomes*, 74.
- Calcium phosphate** deposits in milk, 241.
- Calculi**, Calcium phosphate, in milk, 242.
- Calycella syringa**, 59.
- Campeloma**, 91.
- Campylaspis**  
costata, 63.  
rubicunda, 63.
- Canvas-back Duck**, 182.
- Carbon dioxide** concentration in Ross Lake, 220.
- Carbon monoxide**, Effect of, on *Sclerostomes* in faeces, 203.
- Carbon tetrachloride**, Lethal effect of, on *Sclerostomes* in faeces, 200.
- Carex**, Sedges, as food of ducks and coots, 185.
- Centrovarium lobotes**, 161.
- Chara**, Musk-grass  
as food of ducks and coots, 185.  
Occurrence of, in Ross Lake, 222.
- Charitonetta albeola**, See Buffle-head Duck.
- Chaulelasmus streperus**, See Gadwall Duck.
- Chloride**  
Determination of, in  
cured meat, 134.  
curing pickle, 135.  
in pancreatic juice, 116.
- Chloroform**, Effect of, on *Sclerostomes* in faeces, 200.
- Chloropicrin**, Effect of, on the free-living stages of *Sclerostomes*, 70.
- Cinnamon Teal**, Food of, 182.
- Clangula hyemalis**, See Old-squaw Duck.
- Clinostomum complanatum**, 164.
- Cobalt** supplement, Effect on sheep of, 24.
- Cochlicopa**, 101.
- Cod liver oil** supplement, Effect on sheep of, 22.
- Colorado potato beetle larvae**, Pathogenicity of *Beauveria Bassiana* on, 103.
- Colorimeter**, Photoelectric, used in determination of nitrite and nitrate in cured meat and curing pickle, 128.
- Colostrum**, Microscopic appearance of, 242.
- Colour comparator** to compare meat samples, 2.
- Colour of meat**  
I. Apparatus for its measurement, and relation between pH and colour, 1.  
II. Effect of desiccation on the colour of cured pork, 29.  
Relation to pH, in mutton, pork, and beef, 4.
- Columella**, 101.
- Coots** at Swan Lake, B.C., Food of, 178.
- Copper** supplement, Effect on sheep of, 22.
- Corynebacterium bovis** infection in milk, 232.
- Cow's milk**  
Definition of normal, 230.  
Detection of abnormal, by microscopic methods, 225.
- Crepidostomum**  
brevivitellum, 160.  
cooperi, 158.  
cornutum, 157.  
ictaluri, 158.  
lintoni, 157.
- Cumacea** of the Western Canadian Arctic region, 1936-1937, 62.
- Cumella carinata**, 64.
- Cupric nitrate**, Effect of, on the free-living stages of *Sclerostomes*, 80.
- Cyclopes pygmaeus**, See Anteater, Silky.
- Cyprinus carpio**, 154.
- Dafila acuta tzitzihoa**, See American Pintail.
- Death point**, "Flash" thermal, Apparatus for measuring the, of microscopic animal organisms, 169.
- Decapoda** of the Western Canadian Arctic region, 1936-1937, 62.
- Delicata pseudoappendiculata** sp. nov., 257.
- Deroceras**, 102.
- Derris powder**, Effect of, on free-living stages of *Sclerostomes* in faeces, 203.
- Desiccation**, Effect of, on colour of cured pork, 29.
- Dew-point hygrometer** for use at low temperatures, 35.

**Diastyllis**

- edwardsi, 64.
- goodsiri, 64.
- oxyrhyncha, 64.
- rathkii, 62.
- spinulosa, 64.

**Dichloropentanes**, Lethal effect of, on Sclerostomes in faeces, 196.

**Diets**, Studies with sheep on deficient, 15.

**Diphtheroid-infected cows**, 232.

**Discus**, 100.

**Ducks** at Swan Lake, B.C., Food of, 178.

**Edentates** in Trinidad, Parasites of, 249.

**Endoparasitic fauna** of Trinidad mammals. VI. Parasites of Edentates, 249.

**Erismatura jamaicensis rubida**, See Ruddy Duck.

**Esox**

- lucius as host of trematodes, 167.
- masquinongy as host of trematodes, 167.

**Ethylenedichloride**, Lethal effect of, on Sclerostomes in faeces, 199.

**Euconulus**, 100.

**Exercise**, Muscular, Blood chemical changes during recovery from exhaustive, 109.

**Fasciola hepatica** Linn., Observations on the bionomics of ova and miracidia of, in Eastern Canada, 205.

**Ferric oxide**, Effect of, on Sclerostomes in faeces, 203.

**Ferrissia**, 98.

**Fish**, Freshwater, Parasites of, 154.

**Fontesia fontesi**, 258.

**Food of ducks and coots** at Swan Lake, B.C., 178.

**Fulca americana**, See American Coot.

**Gadwell Duck**, Food of, 182.

**Galba ferruginea**, 211.

**Garbage** fed to hogs, Cooked and uncooked, 151.

**Gastropoda**, 91.

**Gastropods** as food of ducks and coots, 184.

**Gigantorhynchus echinodiscus**, 251.

**Graphidiops costalimai**, 256.

**Greater Scaup Duck**, Food of, 182.

**Green-winged Teal**, Food of, 182.

**Gyraulus**, 98.

**Hawaila**, 100.

**Heligmosominae** sp., 263.

**Hellsoma**, 93.

**Hellebore powder**, Effect of, on Sclerostomes in faeces, 203.

**Hogs**, *Trichinella spiralis* in, 151.

**Horses**, Bionomics and control of bursate nematodes of, 68, 187.

**Humidities**, Colour change of meat in air at different relative, 31.

**Humidity**, Measurement of, at low temperatures, 35.

**Hydrogen ion concentration**

- in Ross Lake, 220.
- Relation between colour of meat and, 3.
- Relation between tenderness of meat and, 12.

**Hydroids** of the Western Canadian Arctic region, 1935-1937, 59.

**Hygrometer**

- Absorption, 35.
- Dew-point, for use at low temperatures, 35.

**Hyperchloremia** in rats after exhaustive muscular exercise, 111.

**Ictalurus punctatus** as host of trematodes, 167.

**Iron supplement**, Effect on sheep of, 22.

**Lacustrine investigations** in the Gaspé peninsula, 212.

**Lampsilis**, 89.

**Lasmigona**, 89.

**Lauroia trinidadensis** sp. nov., 254.

**Leptinotarsa decemlineata**, Pathogenicity of *Beauveria Bassiana* on larvae of, 103.

**Leptostylis**

- ampullacea, 65.
- villosa, 65.

**Leucocyctes** in milk, 229.

- Breed method of counting, 228.
- Sediment method of counting, 228.

**Leucon**

- acutirostris, 63.
- fulvus, 62.
- nasica, 63.
- nasicoides, 62.
- pallidus, 63.

**Linseed meal supplement**, Effect on sheep of, 22.

**Longistriata**

*cristata* sp. nov., 260.  
*urichi* sp. nov., 260.

**Lymnaea**, 92.

**Mallard**, Common, Food of, 182.

**Mareca americana**, See Baldpate Duck.

**Mastitis**

*B. coli*, 238.  
*Corynebacterium pyogenes*, 239.  
*Staphylococcic*, 234.  
*Streptococcic*, 236.

**Meadow nematode**, See Nematode, Meadow.

**Meat**

Colour of  
I. Apparatus for its measurement, and relation between pH and colour, 1.  
II. Effect of desiccation on the colour of cured pork, 29.  
Cured, Determination of nitrite, nitrate, and chloride in, 125.  
Tenderness of: I. A recording apparatus for its estimation, and relation between pH and tenderness, 8.

**Meganema** n. gen., found in the Canadian Arctic, 60.

**Meganema claviformis** n. sp., 60.

**Methylene blue** for staining cells in milk, 228.

**Micropterus dolomieu** as host of trematodes, 167.

**Microscopic methods**, Detection of abnormal cow's milk by, 225.

**Miracidia** of *Fasciola hepatica*, 209.

**Mollusca**, Land and freshwater, from Western Ontario, 87.

**Molluscs**

as food of ducks and coots, 184.  
Behaviour of miracidia in presence of, 210.

**Musculium**, 90.

**Myriophyllum spicatum** as food of ducks and coots, 184.

**Naphthalene**, Lethal effect of, on Sclerostomes in faeces, 195.

**Nematode**, Meadow, *Pratylenchus pratensis* (de Man) Filipjev 1936, Biology of the, 39.

**Nematodes of horses and sheep**, Studies on the bionomics and control of the bursate

VI. On the lethal effects of some nitrogenous chemicals on the free-living stages of *Sclerostomes*, 68.

VII. The effect of some substances, used in the control of farm and household pests, on the free-living stages of *Sclerostomes*, 187.

**Nettion carolinense**, See Green-winged Teal.

**Nicotine sulphate** (40%), Lethal effect of, on *Sclerostomes* in faeces, 197.

**Nitrate**, Determination of, in  
cured meat, 132.  
curing pickle, 133.

**Nitrate reduction** by bacteria in pickling brine, 54.

**Nitrite**, Determination of, in  
cured meat, 129.  
curing pickle, 132.

**Nitrogen**

Protein and non-protein, in pancreatic juice, 116.  
Protein, Relation between absorption spectrum measurements and, 120.

**Nitrogenous chemicals**, Lethal effects of some, on the free-living stages of *Sclerostomes*, 68.

**Nyroca**

*americana*, See Redhead Duck.  
*collaris*, See Ring-necked Duck.  
*marila*, See Greater Scaup Duck.  
*valisineria*, See Canvas-back Duck.

**Oat roots**, Meadow nematode in, 40.

**Oats** and alfalfa hay ration, Effect on sheep of, 24.

**Old-squaw Duck**, Food of, 182.

**Oöchoristica** spp., 251.

**Orthodichlorobenzene**, Lethal effect of, on *Sclerostomes* in faeces, 191.

**Oxygen**, Dissolved, in Ross Lake, 216.

**Pallifera**, 102.

**Pancreas**

Secretion of crystalloids and protein material by, in response to secretin administration, 113.  
Synthesis and secretion of protein material by the, 137.

**Paradichlorobenzene**, Lethal effect of, on *Sclerostomes* in faeces, 190.

**Parasites of**

Edentates in Trinidad, 249.  
freshwater fish. I. Internal trematodes of commercial fish in the central St. Lawrence watershed, 154.

**Pelecypoda**, 88.

**Perca flavescens** as host of trematodes, 167.

**Phagocytosis** of organisms in milk, 239.

**Phenoldisulphonic acid** method for determination of nitrate in cured meat and curing pickle, 132.

**Phosphorus** supplement, Effect on sheep of, 21.

**Photoelectric**

colorimeter used in the determination of nitrite and nitrate in cured meat and curing pickle, 128.  
colour comparator used to compare meat samples at different pH, 2.

**Phyllodistomum**

staffordi, 155.  
superbum, 155.

**Physa**, 99.

**Physaloptera** sp., 253.

**Pickle**, Curing, Determination of nitrite, nitrate, and chloride in, 125.

**Pigs**, Worms of *Ascaris lumbricoides* from, 173.

**Pintonema tamandua** sp. nov., 262.

**Pisidium**, 90.

**Planorbula**, 98.

**Posthodiplostomum minimum**, 164.

**Potamogeton** as food of ducks and coots, 185.

**Potassium** in pancreatic juice, 116.

**Potato beetle larvae**, Pathogenicity of *Beauveria Bassiana* on, 103.

**Pratylenchus pratensis** (de Man) Filipjev 1936, the meadow nematode, Biology of the, 39.

**Protein material**

Secretion of, by the pancreas in response to secretin administration, 113.  
Synthesis and secretion of, by the pancreas, 137.

**Ptychogonimus fontanus**, sp. nov., 166.

**Pudica pudica**, 263.

**Pulmonata**, 99.

**Pyrethrum powder**, Effect of, on Sclerostomes in faeces, 203.

**Pyridine**, Effect of, on the free-living stages of Sclerostomes, 75.

**Querquedula**

cyanoptera, See Cinnamon Teal.  
discors, See Blue-winged Teal.

**Redhead Duck**, Food of, 182.

**Reproduction of sheep** on deficient rations, 19.

**Retinella**, 100.

**Ring-necked Duck**, Food of, 182.

**Ross Lake**, Description of, 213.

**Ruddy Duck**, Food of, 182.

**Sabinea septemcarinata**, 67.

**Salt**, Effect of, on bacteria contaminating sides for Wiltshire bacon, 51.

**Salt resistance** of bacteria on bacon sides, 52.

**Salt tolerance** of bacteria on bacon sides, 52.

**Saponin**, Effect of, on the free-living stages of Sclerostomes, 85.

**Scirpus occidentalis**, Bulrush, as food of ducks and coots, 181.

**Sclerostomes**

Effect of some substances, used in the control of farm and household pests, on the free-living stages of, 187.

Lethal effects of some nitrogenous chemicals on the free-living stages of, 68.

**Secretin** administration, Composition of pancreatic juice secreted under, 117.

**Sediment method** of counting leucocytes in milk, 228.

**Sheep**

Bionomics and control of bursate nematodes of, 68, 187.

Studies with a deficient ration for, 15.

**Shoveller Duck**, Food of, 182.

**Snails** exposed to infestation by *Fasciola hepatica*, 210.

**Sodium** in pancreatic juice, 116.

**Sodium fluoride**, Lethal effect of, on Sclerostomes in faeces, 192.

**Sodium silicofluoride**, Lethal effect of, on Sclerostomes in faeces, 193.

**Spatula clypeata**, See Shoveller Duck.

## — VIII —

- Spectroscopic analysis** of pancreatic juice, 115.
- Sphaerium**, 90.
- Spirogyra** as food of ducks and coots, 185.
- Spirontocaris**  
     *fabricii*, 66.  
     *groenlandica*, 66.  
     *phippisii*, 66.  
     *polaris*, 66.  
     *spina*, 66.
- Stagnicola**, 92.
- Staphylococcus aureus** in milk, 235.
- Stichorchis giganteus**, 250.
- Stizostedion vitreum** as host of trematodes, 167.
- Storage**, Effect of, on colour of meat, 31.
- Streptococcus mastitidis** in milk, 238.
- Striatura**, 100.
- Strobilops**, 101.
- Strongyloides** sp., 251.
- Succinea**, 101.
- Sulphanilic acid- $\alpha$ -naphthylamine hydrochloride** method for determination of nitrite in meat extract and curing pickle, 128.
- Swan Lake**, B.C., Description of, 180.
- Tamandua longicaudata**, Parasites of the, 249.
- Tankage** supplement, Effect on sheep of, 22.
- Tatusia novemcincta**, See Armadillo.
- Temperature**  
     Effect of, on the colour of meat, 32.  
     Lethal, of meadow nematode, 44.  
     observations in Ross Lake, 214.
- Tenderness of meat**. I. A recording apparatus for its estimation, and relation between pH and tenderness, 8.
- Thermal death point** of microscopic animal organisms, Apparatus for measuring, 169.
- Thularia**  
     *similis*, 59.  
     *tenera*, 59.
- Tobacco dust**, Lethal effect of, on Sclerostomes in faeces, 202.
- Trematodes** of commercial fish in the central St. Lawrence watershed, 154.
- Trichinella spiralis** in hogs, 151.
- Trichinosis** in Canada, Investigations on. II. A further survey of the incidence of *Trichinella spiralis* in hogs in Eastern Canada, 151.
- Trichocephalus** sp., 256.
- Trichostrongylidae** sp., 263.
- Trinidad mammals**, Endoparasitic fauna of. VI. Parasites of Edentates, 249.
- Trisodium phosphate**, Lethal effect of, on Sclerostomes in faeces, 200.
- Typha latifolia**, Cat-tail, as food of ducks and coots, 185.
- Udder**, Leucocytic reactions in cow's, 240.
- Valvata**, 91.
- Vertigo**, 101.
- Vietosoma parvum**, 157.
- Vittrina**, 100.
- Wheat bran** supplement, Effect on sheep of, 22.
- Wheat germ meal** supplement, Effect on sheep of, 22.
- Wiltshire bacon**, Bacteria contaminating sides for, 45.
- Zonitoides**, 100.
- Zoogenites**, 101.

## Errata

Page 17, footnote 1, add "Manuscript received October 7, 1938."

Page 211, third line, for "*Gyranlus*" read "*Galba*."





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